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SUBSTANCE OR SYSTEM: THE RIDDLE OF MORPHOGENESIS[1]

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IT has often been said, and truly, that in the problems of morphogenesis, of the controlled formativeness of living matter, center the most important concerns of biological science. A tiny fertilized egg, dividing first in this plane and then that and building up a group of cells which in their growth march steadily on to a determinate organic whole, presents a problem which must ultimately involve not only embryology but most of the other disciplines which deal with protoplasm and its activities. The mechanisms which control this orderly process of development, and which bring it back to normal paths again when accident or experimental change disturb its regular course, are little understood, though since the days of Aristotle the best minds of our science have pondered the problems which are here presented.

To many biologists, aware of the chemical basis of all life and of the profound effects which changes in the chemical environment may produce on living things, it has seemed clear that specific substances must regulate development and control its course. Sachs long ago presented evidence that definite organ-forming substances determine the structures which are produced by plants. This conclusion was strengthened by other facts, such as the important influence on plant growth exerted by particular chemical elements in the soil. Similar observations were reported in animal development, as in the

[1] Address of the retiring president of the American Society of Naturalists presented at its annual meeting in St. Louis in March, 1946.

larva of *Echinus* which is completely altered in its form when grown in an excess of lithium.

With the rise of modern physiology and a better understanding of the biochemistry of growth processes, a more precise relationship could be established between specific chemical agents and the development of an organism. The recognition of hormone activity emphasized this relationship still further. Here are chemical substances which can be isolated and identified and which, even in remarkably small amounts, have the most far-reaching influence upon mammalian development. The effect of the pituitary hormone in controlling body size, of the male sex hormone on body form and other characters, and of the corpus luteum secretion on various organs of the female are familiar examples.

It was not long before similar substances were found in plants. For a score of years or more we have seen the remarkable growth of phytohormone physiology and have learned how the auxins and their synthetic imitators may effect almost all the processes of plant development. Plant hormones have been found to induce root formation, cause ovaries to grow into seedless fruits, stimulate the development of cambium, check the formation of abscission layers, initiate flower formation in higher plants and sex organs in fungi and modify profoundly the form of many plant parts. In these "growth regulators" it seemed to many botanists that we had found at last the key to an understanding of plant development. Indeed, in hypothetical *rhizocalines* and *caulocalines* Went sees specific root-forming and stem-forming substances, a complete return to the ideas of Sachs half a century ago.

Meanwhile on the animal side Spemann and his school developed the hypothesis that in localized regions of the animal embryo there reside abilities to mould the growing structure in specific ways and thus to organize development. With the years this important idea has led to a wide series of researches upon embryonic induction in animals. These have shown that it is not the bit of living

"organizer" which exercises control over development, but specific substances which it contains and which are effective in the absence of a living carrier. The mechanisms by which these substances produce their effects are being eagerly sought by many experimental embryologists.

But modern biochemistry is not to be satisfied with such inexact knowledge as we possess of the substances concerned in the amphibian organizer and of the way in which these mediate the long course of embryonic growth. The hopeful beginning of a more precise analysis of the biochemical basis of development has now been made and in a number of cases it is possible to determine a few of the successive chemical steps which the organism takes in its ontogeny. In that most useful of molds, *Neurospora*, a study of the effect of single gene mutations on the ability of the plant to perform certain chemical changes in its metabolism has made it possible to trace a successive series of such steps and to relate each to a particular gene.

All this suggests that the basis for the control of organic development is a series of chemical substances, each with a specific role, produced by genes and entering in orderly fashion into the ontogenetic cycle. Studies like those which have been made in *Neurospora* have been carried out, though not in such detail, with other animals and plants, and the stimulating possibility is now presented to us of making a complete biochemical analysis of development from fertilized egg to adult and thus of determining the precise chemical correlates of each step in the process. The implication of all this, supported by the considerable body of experimental evidence already mentioned as to chemical control over developmental processes generally, is that organic development in its essence is a series of chemical reactions which result in the mobilization, from materials in the environment, of a mass of living stuff which we call an organism. The specific character of this organism and the form and relationships of its parts are to be attributed to the ac-

tivity of the specific chemical substances which enter into these reactions.

This is the attitude, expressed or taken for granted, which seems to be shared by most students of development to-day. The triumphs of biochemistry already have been so great, its possibility for the future so tremendous and the hope which it holds out for a sound and rational interpretation of biological phenomena so strong and confident that many investigators see no need for any other alternate method of attack on living things and no biological problems which it can not be expected finally to solve. For them, Needham's stimulating book on "Biochemistry and Morphogenesis" points the way to the biology of the future. The more enthusiastic among them, when confronted by a problem in development, seek at once for "a substance" which is responsible for the facts observed. The more cautious realize the complexities of any complete biochemical analysis, but see in it, nevertheless, the only ultimate solution for such problems. The prestige of this ideological position is now so great in many quarters that to challenge it in any important particular is to raise some doubt as to one's scientific judgment.

But the conception of the developing organism as a chain of chemical reactions, each the precursor to the next and each mediated by a specific substance which is itself the ultimate product of a self-multiplicative series of genes, useful though it may be as a hypothesis to provide a front line of attack on developmental problems, nevertheless provides an insufficient answer for the most pressing problem of all in biology and thus has failed to satisfy many thoughtful minds. This problem is the basic one of biological *organization.* The organism is certainly the seat of physico-chemical changes of the most varied sort, but these do not go on independently of each other. They are knit together in such an orderly fashion that an *organism* is produced, an integrated whole, its various parts so correlated in their development that a specifically formed structure results in the normal proc-

ess of development and even succeeds in restoring itself if this normal course is interrupted or altered by experiment. Organic form is the external manifestation of this underlying biological organization. It is not a static but a dynamic thing. The growing organ or body is the seat of continuous metabolic change. The very chemical entities of which it is composed are from time to time replaced by others like them, as experiments with "tagged" atoms have proven. And yet the organization itself, the *system* of interrelated parts and processes, maintains intact its unity and coherence as it marches on through the progressive course of its ontogeny.

This integrated and organized behavior, this unity in the midst of diversity, is the most distinctive feature of life in general. What the mechanisms are which underlie it and what is the physico-chemical basis by which it is established and persists are deep problems which long have vexed students of development. The biochemist for the most part still ignores them, wisely devoting himself for the time being to the immense and productive task of describing and analyzing the many chemical changes here concerned. Occasionally he may speculate, as Holmes has recently done, on the possibility that chemical equilibria and specific steady states may explain organization. Perhaps the answer is not in his field primarily but is to be sought in physical facts rather than in chemical ones. Certainly bioelectrical phenomena offer tempting possibilities. It may be, as Schrödinger believes, that physical principles as yet undiscovered may be operative in living things. The close relationship between biological organization, as manifest in development, and those phenomena which are called psychical, was emphasized by Spemann and has recently been discussed at length by Ralph Lillie. It is clear that this problem strikes deeply into that territory where biology, physics and metaphysics meet, a territory into which only the most intrepid or unwary scientist is likely to penetrate. Such an attempt is far from the purpose of the present paper.

Whatever explanation there may be, however, for this

phenomenon of biological organization, a recognition of its existence is of primary importance for any interpretation of morphogenetic mechanisms. If a living thing is more than a mass of specific substances between which complex reactions are continually taking place; if it is, in its ultimate essence, an organized system into which these substances and processes are integrated, then our ideas of the manner in which it develops and undergoes modification must take into account this profoundly significant fact. It points to the conclusion that the effects which a specific substance or any other physical factor may have upon the organism are not primary ones, leading directly to specific morphogenetic consequences, but that these factors are simply *evocators* acting upon the organized system which, by its particular response to them, produces a morphogenetic result. What is formed is not simply a chemical product, the end result of a chain of reactions, but a fabric of living material regularly distributed in space and time. A specific substance certainly may greatly modify this distribution and thus affect organic form; but the way it does this is perhaps suggested by analogy with the hypothetical forces which deform the coordinate systems in which D'Arcy Thompson inscribes organic profiles, where the whole system is modified and no single part alone. The fact that auxin has been found to affect so many very diverse structures and processes indicates that what it does depends upon the system in which it is operating. Specificity inheres not in the evocator, but in this organized system from which it calls forth a reaction. The organizer postulated by Spemann, though great hopes were had that it might be something more, proved at last to be only a complex evocator releasing a specific reaction. As Spemann says: "The share of the system of reaction turned out greater and greater, so great at last that the very conception of the organizer became problematical."

Not all the specificities, of course, not all the problems to be solved, reside in the organized reactive system. Evocators differ in character and effect. The various

synthetic growth substances for plants have many general resemblances but are by no means identical in their results. Some are particularly effective as root inducers, others in producing parthenocarpy, and others as stimulators of growth so abnormal that it ultimately is lethal. Similar differences may be observed in other physiologically active substances which are receiving attention from students of development. Their precise character is indeed important, but far more significant is the organized system upon which they work. In studying them we are somewhat in the position of the enthusiastic student of coin physiology who might examine the character of nickels, dimes, cents, quarters and divers imitations of these to find how they produce their various results in the familiar mechanisms for which they are employed. A nickel, they would discover—and sometimes a slug!—will activate a dial telephone, open a subway turnstile, call forth a tune from a juke box, or provide an hour on a parking meter. A cent will not do these things, but it will obtain a piece of gum from a vending machine or a ticket with one's weight stamped on it from an automatic scales. A quarter is useless for these purposes, but possesses unique capacity for opening a gas meter. There is a certain specificity in these coins, but it is obvious that an explanation of the results which they accomplish will come not from a more detailed study of the coins themselves but of the complex mechanisms which they activate. There is here, I believe, something more than a rough analogy with the processes of morphogenesis. No analysis, however complete, of substances known to have morphogenetic effects will explain their results. Only a knowledge of the organized systems upon which they act will solve the problem.

This conclusion may appear so self-evident to many as hardly to be worth repeating, especially in a decade which has witnessed so many proofs of the complexity of biological systems. The dominance of the biochemical point of view in developmental problems to-day, however, naturally leads to such an emphasis upon specific substances

and reaction chains that we sometimes lose sight of the even deeper problem of what it is that binds these into an integrated system. Modern biochemistry has already won such notable triumphs and stands so certainly upon the threshold of vast new discoveries that it has captured the imagination of the rising generation of biologists. There is a danger here, perhaps, that our students may come to believe that nothing is really important in biology unless one can write its structural formula. This could lead to the same sort of overemphasis which all science suffered half a century ago, when nothing seemed significant in biology which was not related to the great problem of reconstructing the phylogenetic system of the organic world. Science grows by these intense concentrations upon particular objectives. But as teachers we should strive to keep the whole biological program before our students if they are to deal constructively with the ever-widening range of problems which the future must meet. Certainly the biochemical attack must be pressed with all possible energy. It is the dominant biological discipline of the day. But we should strive to keep it from slipping into a narrow materialism which would shut its eyes to a wide range of life phenomena and belittle their importance because it can not account for them. The triumphs of biochemistry in tracing the series of changes which lead to the production of specific substances under the control of genes or which have begun to clarify the complex processes of respiration still are far from approaching the fundamental problem of biological organization and the control of development as a whole. It may well be that the chief significance of biochemistry will continue to be primarily in the elucidation of metabolic processes and that a strictly chemical attack will never be able to explain development. Many of its protagonists, however, hope and believe that it will, and look to chemical equilibria or molecular configuration or some other chemical fact which may yield the secret of organic form. Experience has shown how difficult it is to read the future of science and how often those events which were confi-

dently said to be impossible have actually come to pass. Nevertheless, it seems unlikely to-day that biochemical discoveries, fruitful as they are sure to be in many fields, will be able to explain the precise spatial distribution of diverse substances which accompany the development of organic form and the differentiation of its parts in an organized pattern. Whatever the answer of the future is to be on these questions, the student of morphogenesis should certainly endeavor to keep his mind open to clues from any direction, even most unlikely and unorthodox ones, which offer any prospect of answering the deep problems with which he is concerned. Specific substances and their effects he certainly will examine. But to the present writer it seems more likely that ultimate success here will follow an attack conducted primarily from the direction of biophysics rather than of biochemistry. Both these disciplines ultimately deal with the same phenomena, but the concern of biophysics with the distribution and patterning of matter rather than with its specific character seems to offer more hope for an understanding of the inherent formativeness and organizing capacity displayed by living stuff.

In conclusion, then, I would say that for developmental problems a study of substance is important but that the ultimate question is rather the organized system in which this substance operates. This is no time, however, to limit ourselves to any single approach. Biology is still so far from certainty in its deeper concerns that we should neglect no possible avenue of approach to new discoveries. Even its more mature and traditional fields are far from being overworked and will still yield a rich harvest to those trained to reap it. Let us try, in these days of intense specialization, to cultivate more of the catholicity of the earlier practitioners of our science, and especially to implant in our students an interest in and respect for all the branches of biology. From somewhere in this wide field—perhaps from where we expect it least now—may come the next new unifying idea which will show how system relates to substance and how life depends on both.

ON THE MECHANISM OF HETEROSIS—THE CHROMOSOMAL OR CYTOPLASMIC BASIS FOR HETEROSIS IN *DROSOPHILA MELANOGASTER*[1]

DR. JOHN W. GOWEN, JANICE STADLER AND DR. LESLIE E. JOHNSON

In 1937, work on evaluating the relative contributions of chromosomal and cytoplasmic factors to the vigor observed in race crosses was begun in this laboratory. A race of flies, inbred single pair, brother by sister for 37 consecutive generations, was chosen for the experiments. The expected high homozygosity—something greater than 99.9 per cent. on the bases of a rather formidable list of assumptions, that mutation, linkage and selection effects were negligible—made this race attractive as foundation material. The question of primary interest was: Does such a highly inbred stock contain or develop the requisite variation necessary to establish new or genetically different races in subsequent generations?

Three methods of breeding were adopted. In the first, the inbred stock was continued by random mating over a period of 10 generations. In the second, the inbred stock was continued by further sib mating, one pair for each generation. In the third, the 37th generation sib inbred race was divided into sub-races, each of which was made "homozygous" for the first three chromosomes, by an outcrossing technique found in Fig. 1.

The observed data were unorthodox. Their pertinence to much in genetic theory and to the validity of mathematical models which have been set up to calculate genetic behavior made it desirable to put the observation on a broader genetic base. Subsequent experiments have involved races of widely different geographical and genetic origin. We shall review the experiments separately, as each has unique features.

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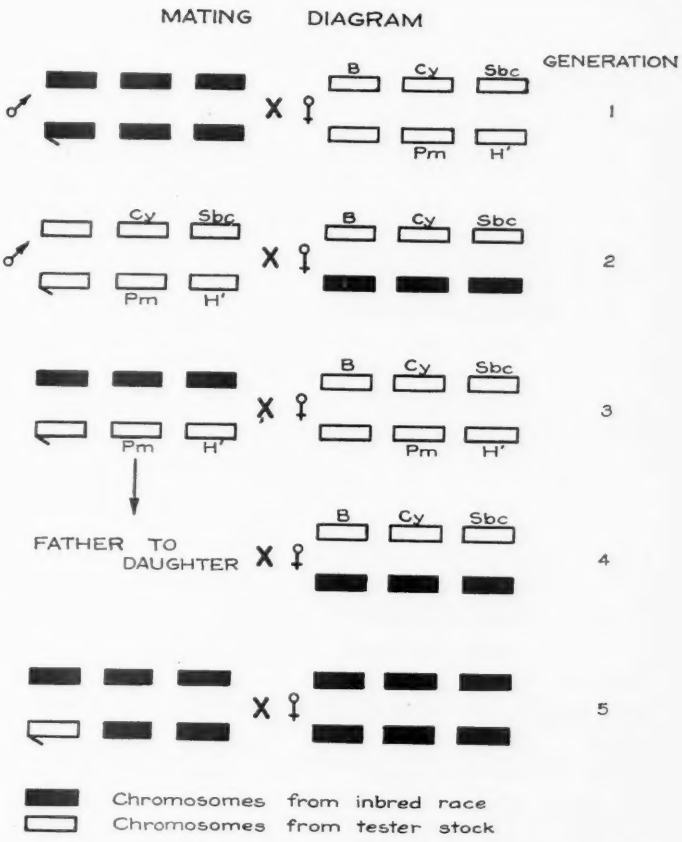

FIG. 1. Diagram of matings to form "homozygous" lines. The chromosome order is I, II and III. The solid chromosomes come from stock to be made homozygous. The open chromosomes are of test stock origin. The C1B chromosome contains the genes sc t^2 v sl B and a lethal. The inversion in this chromosome is between 3E2 and 17A4. The Cy chromosome contains an inversion in left arm of chromosome II, 22D1 to 33F and another in the right arm, 42A2 to 58A. The Pm chromosome contains dp b Pm and an inversion from 40F to 59E1; the tips 2L and 2R are interchanged, left break at 21D1 and right at 60D1. The Sb chromosome contains gene e^s and inversions in left arm of chromosome III, 63B1 to 72E1 and right arm, 92E1 to near end. H is normal marked chromosome containing Hairless gene.

Material and Methods

The foundation stock of the first experiment came from H. H. Plough of Amherst, Massachusetts. It had been bred single pair, brother by sister for 23 generations prior to our receiving it. We continued the matings in the same manner to the 37th generation. We designate this race as Inbred 37. In the 37th generation the progeny was divided at random into 3 lots. One lot was again divided into 2 lines, each of which was continued separately for 10 generations by random mating of 60 flies per generation. They are designated as Random bred. The behavior of this group furnishes a measure of the variability of the original 37 generation inbred parental stock from which the experiments were started.

A second of the 3 lots was subdivided into 35 new inbred strains, each of which was continued by brother by sister pair matings for another 10 generations of inbreeding, 47 generations in all. Only 2 of the 35 strains were lost in the 10 generations. These losses were probably accidental.

The third lot of flies was bred by the outcross technique illustrated in Fig. 1 in an attempt to produce strains homozygous for the first, second and third chromosomes. Eighteen strains were produced. With perfect conditions each strain would be homozygous for chromosomes I, II and III. Variation between strains would be due to residual variability yet remaining in the parent 37 generation inbred race or to IV chromosomal effects. About 98 per cent. of the total chromatin would be controlled, the small 4th chromosome being the only portion of the chromosomal inheritance disregarded. This is theoretical, however. Practically the inversions for preventing crossing over are not as perfect as could be wished. When all 3 chromosomes have inversions the amount of crossing over is noticeable. Some of this crossing over may be detected and the progeny discarded, but some could escape detection. We regard these strains only as "homozygous" as care and the crossover preventor will

allow. Better inversions and marker stocks were used in subsequent stages of this work, but these too have their difficulties. However, actual crossing-over tests with these chromosome stocks show very little undetected crossing over. Fig. 1 is a diagram of the breeding techniques.

Later experiments used the marked inversion stock indicated in Fig. 2. The improvement comes largely in using more recessive genes at the ends of the chromosomes. These recessives will detect crossovers which might occur and be otherwise unobserved. For those who might care to follow this technique we might say that it is beset with pitfalls and has marked possibilities of improvement. Particularly it needs good dominants in the 4th chromosome which will not conflict with those available in other chromosomes. It needs genes without complementary effects detrimental to viability.

The techniques described in Figs. 1 and 2 make possible the production of "homozygous" individuals by outcrossing. The cytoplasm of the original race is replaced by that of the tester stock. As this tester stock is itself maintained by outcrossing, it follows that this cytoplasm is comparable with that of any wild race in being from fully vigorous parents with heterogeneous cytoplasm. This cytoplasm may then be contrasted with that of the highly uniform cytoplasm which should be present in a race whose ancestry has been long and intensively inbred.

Genes in the chromosome pairs of the "homozygous" lines should be closely similar. They should be quite like those of the donor inbred race from which they started, save that they would be homozygous in even those loci which may have remained heterozygous under close inbreeding. The contrasts are rather obvious; if heterosis is due to cytoplasmic differences, even then the "homozygous" stock with its heterogeneous cytoplasm should have greater vigor and yielding ability than that of the inbreds. If heterosis is due to gene differentiation within loci then the inbreds with the greater heterozygos-

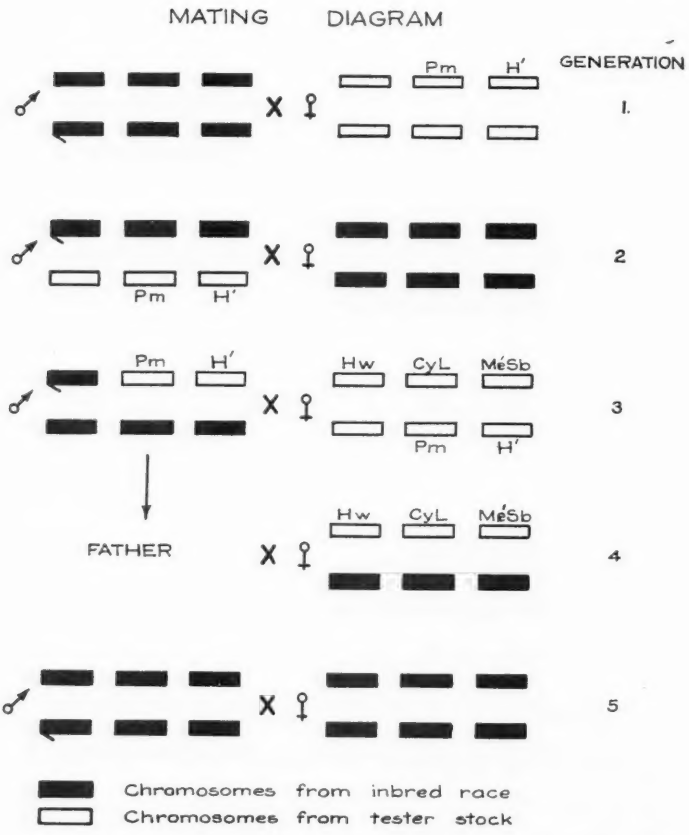

FIG. 2. Diagram of matings to form later "homozygous" lines. Significance of chromosomes that of Fig. 1. Hw chromosome contains the genes y Hw t^2 v g^2, with a lethal and an inversion, 3E2 to 17A4. Cy L chromosome contains al^2Cy lt^3 L^4 sp^2, inversions in left arm, 22D1 to 33F, in right arm 42A2 to 58A. Pm chromosome contains dp and b and inversion 40F to 59E1, with tips of chromosome interchanged at 21D1 and 60D1. The Mé Sb chromosome contains e^s and ca, inversions 63B1 to 72E1 and 92E1 to near end of chromosome. H chromosome has gene Hairless but is otherwise normal.

ity should show greater productivity than the "homozygous" strains. Any differences in production between the 2 categories depend in part on the residual

heterozygosity held within the inbred group. A test of this heterozygosity of the Inbred parent race after 37 generations of brother by sister pair matings is had as one of the by-products of the technique by which the homozygous types are made.

Heterozygosity of 37th Generation of the Inbred Race

Selection for normal types as parents of each generation of the Inbred race has eliminated any visible mutants or other overtly divergent phenotypes which may have appeared. This selection, combined with intense inbreeding, tends to prevent any divergence from the pattern of the wild-type fly. The possibilities of a heterozygote continuing in the race seem limited to recessives with no obvious effect on the heterozygote. Of such genes, the class recessive lethals appear most likely of preservation. A single lethal in the population inbred would be highly selected against in the homozygote, a selection so intense that seemingly it could not remain in the population for more than a few generations. However, should another opposing lethal appear in the other chromosome of the pair the chance of survival for both lethals would be greatly increased. This second lethal would then tend to balance the first and continue the heterozygote in preference to the homozygote. The genes within the balanced system would then be free to vary as they would, making for increased heterozygosity in spite of the continued inbreeding. Progress toward homozygosity would be at a standstill. The mathematical model so often used in considering inbreeding effects on genotype would fail. The frequency with which such events occur becomes important in understanding the problem.

Thirty-seven flies were selected at random from the parent Inbred race as donors for "homozygous" lines. Each line was mated according to the plan in Fig. 1 for the five necessary generations. In the process seven lines were lost by proper breeding types not appearing or through accidents. This loss was probably random so

far as any variability or differentiation of the original 37th generation donor chromosomes were concerned.

Twelve or 40 per cent. of the remaining 30 original donor flies were definitely heterozygous, as they demonstrably contained recessive lethals. Lethals had not been eliminated in these flies even though they had been brother by sister mated for 37 generations.

Eighteen of the strains were established as "homozygous" lines. Many of these 18 lines showed small but clear-cut differences in size and general form, again indicating that heterozygosity had remained within the inbreds. The living form was definitely more complex in its behavior toward the inbreeding than the simple mathematical model and as such the results from the model strictly applied would lead to fallacious conclusions.

Several of the new "homozygous" strains were highly sterile. One was lost before it could be tested. Again diversity in the genotype of the parent inbred is indicated.

The 12 lethal-containing strains were tested for location of the lethals. All 12 strains had lethals in the third chromosome. Seven had lethals in the second chromosome. The manner in which the lethals were kept was such as to prove that the lethals had come from the inbred stock and not in some unlooked-for manner, from the tester stocks used in making these strains "homozygous."

The lethal strains were tested against each other to see if they contained an identical lethal or a different one. Between 50 and 200 progeny were counted for each cross. If all or nearly all progeny contained the dominant marker genes the conclusion was drawn that the lethals were the same. If a significant number of wild-type flies were present, then the lethals contained in the two parents were considered different. The breeding methods used in these tests are as follows. The lethal-containing strains tested are known to have their lethals in the second chromosome. The lethals in such strains are kept as the lethal-containing chromosome balanced by the in-

version chromosome Curly. Mating two such stocks together gives progeny in these expected numbers; one lethal I/lethal 2 as wild phenotype, one lethal I/Cy and one lethal 2/Cy as 2 Curly phenotypes. The expected Cy/Cy type dies due to the lethal in the Curly test chromosome. The expected ratio is thus 1 wild type to 2 Curly if the lethals are different. If the lethals are identical, only Curly phenotypes generally appear, the wild type being killed off by the homozygous lethal unless rare crossing over takes place. The data are shown in Table 1.

TABLE 1

PROGENY FROM MATINGS OF LETHAL STRAINS

Second chromosome matings	Mutant marked progeny	Wild-type progeny
9 × 14	124	0
14 × 23	85	2
23 × 26	129	1
26 × 33	101	0
33 × 37	102	0
37 × 13	170	0
Third chromosome		
7 × 9	75	0
8 × 9	84	0
9 × 13	124	0
13 × 14	125	1
14 × 16	13	1
16 × 23	58	0
23 × 26	197	4
29 × 33	64	0
33 × 36	46	24
36 × 37	69	29
37 × 7	56	0

The lethal strains picked up by this technique are interpreted as being the same lethal in the second chromosome and as two different lethals in the third chromosomes. Eleven strains contained one lethal and one strain, number 36, had the other.

This ratio of the detected lethals has a bearing on their arrangement in the parent race. If the parent race actually contained flies bearing 2 lethals, one in each of their second chromosomes and thus in balanced condition for continuance in all future inbreeding, then the chance of obtaining only one of the lethals out of 6 trials would be a^6 or b^6 from the relation $(a + b)^6$ where a and b each equal $\frac{1}{2}$. The chance is thus 2/64. An arrangement of

two balanced lethals would thus seem rather unlikely. For the third chromosome the chance of obtaining 11 or more of one lethal out of 12 trials would be 26/4096 or the balanced arrangement would appear even more unlikely on chance alone. However, in this experiment we did obtain 2 different lethals which in itself lends credence to the tendency for inbred races to collect and balance their homologous chromosome pairs with lethals, thus retarding further progress toward homozygosis in continued inbreeding.

Two sets of matings were carried through to locate the lethals in the 3rd chromosome. Crosses were made to the tester stocks ru h st e^s/ru h st e^s and st ca/st ca. From the backcrosses, the appropriate single crossovers were chosen and tested for the presence of the lethal. The data are too voluminous to warrant publication in detail. Both lethals were in the right arm of the 3rd chromosome. Three crossovers between scarlet and claret, 1 scarlet and 2 claret, for one lethal indicate that its locus is near the midpoint between scarlet and claret. One crossover for the other lethal would favor it nearer claret. This information is tenuous, but is as far as available tester stocks and the significance of the locations seemed to warrant carrying the tests.

Both lethals appear to be associated with short inversions as crossing over between scarlet and sooty is reduced. This might suggest that the lethals were in some unlikely manner derived from the tester chromosome during the cross. However, this possibility seems closed as we indicated since we carried these lethals over many generations balanced by the indicated tester chromosomes, thus showing that allelic lethals did not exist in the donor and tester chromosomes. No cytological study of the chromosomes was made.

The results demonstrate that highly inbred races may carry a fair amount of semi-permanent diversity in their germ plasms. The high frequency of the third chromosome lethals may come as a result of a recent mutation.

But if this is the explanation it seems that the heterozygote must have some selective advantage in order that the lethal may withstand continued brother by sister mating. Rather it appears more likely that in inbreeding there is a strong tendency for lethals to balance one another, thus forming a store of ever-growing variability.

The significance of these observations was considered sufficient to warrant repeating the experiment with this same race. Tests for lethals were made on progeny coming from 20 generations of random mating following the 37 generations of brother by sister mating. Twenty-nine individuals were analyzed. Of this number 7 died without progeny, one did not have the second chromosome tested. Of the second chromosomes tested, 8, or more than one third, contained lethals. Of the 22 third chromosomes tested, the astounding number of 21 had lethals.

On such data the view seems forced that lethals in presumably random breeding populations are capable of not only maintaining themselves in the germ plasm of the population but of increasing their number. This favorable breeding balance for the lethal in heterozygous condition, despite the disgenic effects when homozygous, must mean a favorable survival value for the heterozygote. The long, inbred stock here used should have fair genetic variability between alleles as contrasted with the "homozygous" stock.

Genetic and Cytoplasmic Effects of Inbreeding versus Outbreeding

As described earlier, strains were made up from the Inbred 37 race to conform to 3 different breeding systems; random mated for 10 generations; inbred for 10 generations further, 47 in all, brother by sister; and "homozygous" by outcrossing. Egg production[2] was chosen to measure the effect of these breeding systems on

[2] See Section 1 of this series, "Metabolic Capacity of Different Races of *Drosophila melanogaster* for Egg Production," by John W. Gowen and Leslie E. Johnson (Am. Nat., 80: 788, 149–179, 1946), for methods.

the general vigor and well-being of the progeny. Twenty females were tested for each breeding system or 60 females for an experiment. The experiments were repeated twice. Daily laying records over 5 consecutive days were kept for each female. The mean egg productions per day for the different breeding systems are shown in Table 2.

TABLE 2

MEAN EGG PRODUCTIONS FOR EACH BREEDING SYSTEM. 40 FEMALES IN GROUP

Breeding system	Egg yield per day
Random mating 10 generations following 37 of brother by sister ...	33.0
Inbreeding 47 generations brother by sister	27.4
"Homozygous" by outcrossing	21.2

The results show that the original stock, inbred 37 generations prior to these experiments, is still capable of further modification.

Inbreeding maize has shown that continued close mating results in a decline in corn yield. The data on corn have generally not extended beyond the 10th or 12th generation of selfing. In the data of Table 2 we have a like parallel for Drosophila egg production. When brother by sister mating was stopped at the 37th generation and then was followed by 10 generations of random mating, the egg production was distinctly higher than that for females which had 10 further generations of inbreeding. Either the yielding capacity of the 37th generation had been subject to further modification or the 10 generations of random breeding had developed further variability resulting in the higher yield.

The strain made "homozygous" by outcrossing gives a practical answer to these questions. The "homozygous" are distinctly lower in productions than the inbreds. In fact, the results are a graded series. The strain having the greatest opportunity to be heterozygous metabolizes the greatest amount of energy as eggs; the closely inbred strain with its presumably greater homogeneity shows an intermediate egg yield; the "homo-

zygous'' strain with its possible homozygosity for the great majority of its genes shows the lowest production. The results would satisfy those who interpret the decline in vigor and productivity of inbreeding to the continued increase in like alleles as this system of mating is continued.

Two different systems of matings were used in increasing the number of like alleles within the particular strain; inbreeding with its consequent increasing homogeneity of gene and cytoplasmic effects; outcrossing homozygosity with the effects limited to the genes, the cytoplasm having been replaced by outcrossed heterogeneous cytoplasm at each cross.

The analysis of the effects of hybrid vigor has, up to the present, had but one of these systems of matings from which to draw information, the inbreeding system. Information from the system has led to two major possible interpretations of the great increases in yield and vigor brought about by suitable outcrosses of highly inbred lines: (1) The increase in yield may be due to changes in genic interaction brought about by the gene differences introduced in the cross. (2) The increase may be due to a change in the cytoplasmic reactions set up through combining different cytoplasms.

In principle, the data of Table 2 furnish a fairly critical answer to these questions as the introduction of the ''homozygous'' stock furnishes unique evidence. Under the theory that uniformity of the alleles leads to reduced yield the ''homozygous'' stock should have a smaller yield than the inbred stock, since more of its alleles should be identical. Under the theory that a uniform cytoplasm is detrimental, the ''homozygous'' stock should show a higher yield than the inbred stock since its cytoplasm is quite different from that of the highly inbred strain. The results are evidently in favor of genic behavior as the cause of hybrid vigor as the yields of the ''homozygous'' strains are distinctly less than those of the inbred races. The significance of the results is, however, depen-

dent on their variability. The variance analysis is given in Table 3.

The comparison of the variation within females, strains, systems and experiments, 174, with that between females within strains, systems and experiments, 470, shows that despite the inbreeding of the original stock there is more variation between females than between the daily egg records within single females. Significant individual differences of hereditary or environmental origin thus remain between females.

The comparison of the mean square between strains of females within experiments and systems of mating,

TABLE 3

VARIANCE ANALYSIS FOR THE PROGENY OF RANDOM, INBRED, AND "HOMOZYGOUS" SYSTEMS OF MATINGS, 120 FLIES WITH 5 DAILY EGG RECORDS AT MAXIMUM PRODUCTION

Sources of variation	Symbol	d/f	Mean square	Components of variation
Between:				
Experiments	E	1	12159	5F + 23.3S + 100 EG + 300 E
Random, Inbred, Homozygous	G	2	6990	5F + 23.3S + 100 EG + 200 G
Experiment × mating system	EG	2	1905	5F + 23.3S + 100 EG
Strains of females within experiment and system	S	38	1079	5F + 12.1S
Females within strain, system and experiment	SF	76	470	5F
Remainder		480	174	F

1,079, measures the possibility of isolating the variance of the different females into self-reproducing strains. The chance expected mean square for this variance would be 470 or for 38 and 76 degrees of freedom the strain differences are in the highly significant class. The total evidence consequently favors some residual hereditary influence, yet remaining within the original 37 generation brother by sister mated stock.

The random group was carried as two separate lines for ten generations. Differences in this system were not significant. The inbreds were carried as ten separate lines. The differences among these ten lines were significant at the 5 per cent level. The "homozygous" cate-

gory comprised ten lines. The differences of these ten lines were significant at the 1 per cent level. The effects of the breeding systems conform to the intensity with which each system should lead to homozygous types. Random mating should not and did not lead to line differentiation. The close inbreeding should tend to set the lines further apart according to the amount of residual variation held in the original 37 generation Inbred stock. This the inbreeding system did, but not as completely as the outcrossing system designed to lead to homozygosis. The "homozygous" lines were the most extremely differentiated.

These results show that the 37 generations of brother by sister matings still left possibilities for variation within the stock. This variation is over and above that observed in the analysis of this stock for lethal genes.

The most interesting comparison for our problem is the mean square between the three breeding systems, random, inbred and homozygous, 6,990, and its error mean square. Unfortunately the three groups were not equally balanced for numbers; the random bred strains were two, the inbred and homozygous ten each. The interpretation of the mean square is affected by this unequal weighting.[3] The change comes in the coefficient of S, the strains of females within experiments and systems contributing to variance. The value of this weight is $1/38\left[N-\Sigma\left(\frac{\Sigma k^2}{\Sigma k}\right)eg\right]$ or 12.1. The weighting of S in the EG mean square is $1/2\left[\Sigma\left(\frac{\Sigma k^2}{\Sigma k}\right)eg-\Sigma\left(\frac{\Sigma k^2}{\Sigma k}\right)G-\Sigma\left(\frac{\Sigma k^2}{\Sigma k}\right)E+\frac{\Sigma k^2}{N}\right]$ or 23.3. The weighting for G is $1/2\left[\Sigma\left(\frac{\Sigma k^2}{\Sigma k}\right)G-\frac{\Sigma k^2}{N}\right]$ or 23.3. For experiments, E, the weighting is $\left[\Sigma\left(\frac{\Sigma k^2}{\Sigma k}\right)E-\frac{\Sigma k^2}{N}\right]$ or 23.3. These values are included in the equations interpreting the various mean squares.

[3] In this analysis we are indebted to Dr. G. E. Dickerson for his advice.

The experiment by mating system mean square, 1,905, is not significantly different from its error term 1,079 either before its correction or after the adjustment for the difference at the S coefficient, 1,688. These results may be combined to form the error term for between systems of mating mean square 6,990. This error mean square is 1,699. The F value is 4.1 or the P is about 103. The interpretation of the difference in production of these three systems of breeding as important is thus justified.

These data cover problems of such fundamental interest that it seemed wise to check them rather thoroughly. The further investigations are summarized below.

Further Investigations of Hybrid Vigor

The comparisons were limited to two breeding systems, inbreeding and outcrossing to make "homozygous" types. The stocks used to form the "homozygous" races had much better crossover prevention and detection than those described and used in the experiments above. The technique is presented in Fig. 2.

Eight inbred races and their "homozygous" counterparts have been saved and tested as the result of this experiment. Two of these races stem from wild stock collected originally in Florida and New Jersey. The Inbred race is of the same origin as previously described. The origin of these stocks is described in the first paper of this series (Gowen and Johnson, 1946). The further significant facts are as follows:

Princeton 1 inbred was mated brother by sister using 5 males and 5 females for 14 generations. It was then pair-mated for 9 or more generations. At the 23rd generation the outcrosses for the formation of the "homozygous" Princeton 1 were commenced. By the time these were completed and the "homozygous" Princeton 1 obtained, the inbred had been pair-mated, brother by sister, for 5 more generations. The comparisons

are made between the inbred and the "homozygous" Princeton.

The Princeton 8 and 10 selections originated from independent pair selections from the original random bred Princeton stock. Each was mated 5 pair for 14 generations, then single pair, brother by sister for 14 generations more. At the 28th generation, individual flies were selected for outcrossing and formation of the "homozygous." Comparisons of the "homozygous" and inbred Princeton lines were made on 15 generations of strict brother by sister mated stock.

Florida 2 came from our Florida random bred race. Its breeding history was similar to that of Princeton 1 line.

Florida 5 had a like history save that it originated from the 5th pair selected from the random mated Florida race. It was then split into separate lines numbering 8, continued by brother by sister mating.

Inbreds 3, 4 and 9 all represented selections from our original brother by sister, pair mated Inbred race described earlier. The start for the "homozygous" was the 97th generation of the single pair, brother by sister of the present race. The breeding technique was similar to that of the other races described above.

The formation of these lines allowed us to test the adequacy of the mathematical hypothesis that a system of brother by sister mating leads to homozygosis in the lines which diverge from the parental stock. For this purpose the lines were examined for lethals in the I, II, III chromosomes.

We were able to make the tests for lethals on some of these lines. One came from the Princeton race, 1 from Inbred and 2 from Florida. The results are shown in Table 4.

The lethals observed were all in chromosomes II and III. Princeton 1 had 5 individuals with at least one lethal in chromosome II and 2 with a lethal in chromosome III out of 16 isolations. All 17 isolations from

TABLE 4

GENOTYPES OF INBRED RACES FOR VIABLE, LETHAL AND RECESSIVE VISIBLE ALLELES

Race	Line	No lethal	Lethal	Recessive visible	Total isolation
Princeton	1	9	7	0	16
Inbred	9	0	17	0	17
Florida	5	98	20	2	118
Florida	2	0	12	2	14

Inbred 9 had lethals in chromosome II. Florida 5 had 10 lethals in II and 10 in III out of 118 isolations. Florida 2 had 8 lethals in II and 4 in III in 14 isolations. Lethals in chromosomes II and III were found simultaneously in 4 of the isolations; 2 from Princeton, 1 from Inbred and 1 from Florida. The 4 visible recessives were located in chromosomes I, III and IV. The mutation in chromosome I, forked, had just occurred as but 1 individual in 64 flies was observed. The 2 recessives in chromosome III were a humpbacked thorax and a 4 sex combed, broad-spread-winged mutant with other modifications. The 4th chromosome recessive had the legs, particularly, the anterior, shortened to stumps. Photographs of these mutations are shown in Fig. 3. All these genes were semi-lethal in the larval stages. The humpback had fair viability in the adult, but the other 2 were shortlived.

The known unlike chromosomes held within these brother by sister pair-mated races range from 17 per cent. to 100 per cent. If we accept the mathematical model so often employed in inbreeding calculation we come to see in these results the significance attached to the postulate that true random breeding shall exist. Without it, and reproductive processes seem to have ways of avoiding it, the conclusions drawn from the model are misleading.

Pressure of other work did not allow us to test all these racial lines for the identity of the lethals. We did test the lethals from the 118 Florida isolations. They were distributed between the lines as follows, the line being given first, followed by the lethal isolations; Florida 3–2, 4–2, 5–2, 8–2, 9–1 and 10–1 for lethals in the second chro-

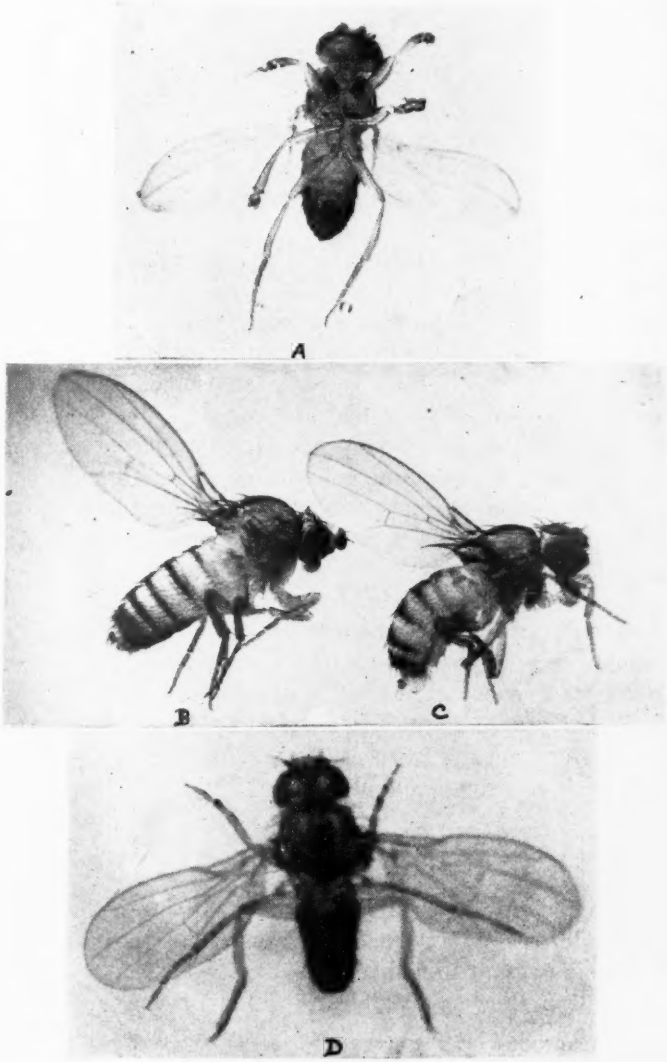

FIG. 3. Photographs showing extreme phenotypes of mutants derived from inbred races.

mosome. For the third chromosome the lethal isolations were Florida 1–5, 3–2, 6–1, 8–1 and 10–1.

For the second chromosome lethals the 2 from line 3 were identical. The 2 from line 4 could not be tested against each other as one died without progeny. The 2 from line 5 were identical. But the 2 from line 8 were different. A balanced lethal arrangement is indicated for line 8.

The third chromosome in Florida 1 line had 2 of the 5 isolations with different lethals. One of these lethals was identical with another Florida 1 isolation. We were unable to complete the tests with the 2 remaining Florida lethals. The 2 Florida 3 third chromosome lethals were untested as one of them had no progeny. The evidence here is like that of chromosome II, balanced lethals are on their way to becoming established in each line.

Of the second chromosome lethal isolations tested, two had identical lethals occur twice and one had identical lethals occur 3 times. For the third chromosome the same lethal appeared in 4 different isolations. These facts are evidence for these lethals being in the original random bred stock from which the pair mated, brother by sister lines were derived 28 generations earlier.

The above evidence for biological mechanisms to maintain heterozygosity in races, even though they are closely inbred, seems unmistakable.

Genetic and Cytoplasmic Effects of Inbreeding vs. Outcrossing

The genetic and cytoplasmic effects of inbreeding versus outcrossing are further evaluated in the data and analysis present in Table 5.

Table 5 shows paired tests of the progeny resulting from brother by sister matings in a given race compared with those which are made "homozygous" by outcrossing. While the results are not wholly unequivocal, the weight of data does point in the same direction as that of the earlier experiments. Each line had its inbred and

"homozygous" tested concurrently. Repeat tests were made at different times and therefore under different conditions. Each test is regarded as a separate experiment. There are 8 races for which inbred and "homozygous" are compared; comparisons within a race range from 1 to 4.

When the mean egg production of the "homozygous" is less than that for the inbred it is indicated by the minus sign, when greater by plus. Thus, for the first test on Princeton 1, the inbred progeny resulting from the brother by sister matings for 28 generations had an egg production of 73.6 eggs per day over the test period. The "homozygous" coming from the outcross breeding system had an average egg yield of 62.7 eggs or the "homozygous" production was 10.9 eggs less than the inbred.

The differences for the different inbred—"homozygous" comparisons ranged from −22.0 to +10.6 eggs. In 19 comparisons, the inbreds produce more than the

TABLE 5

VARIANCE ANALYSIS FOR PROGENY OF INBRED (BROTHER BY SISTER) AND OUTCROSS (LEADING TO HOMOZYGOSIS) MATING SYSTEMS

Race		Mean difference	Days	Breeding systems	Days × systems	Females within breeding systems		Days within females and breeding system		value F
						d/f	*M.S.*	*d/f*	*M.S.*	
Princeton	1	−10.9	5784	8430	949	168	1420	672	150	5.9*
Princeton	1	− 2.8	820	366	3025	168	1319	672	180	.3
Princeton	1	− 9.9	2541	5536	1171	54	1504	216	194	3.7
Princeton	8	+ 6.6	5071	1774	285	48	840	192	118	2.1
Princeton	8	− 6.3	369	1279	89	24	439	96	220	2.9
Princeton	8	− 1.3	638	53	210	23	635	92	199	.1
Princeton	10	+ 6.0	8330	1578	230	78	823	312	106	1.9
Princeton	10	+ 0.1	754	1	912	23	298	92	172	.0
Princeton	10	−15.9	247	9520	179	28	320	112	157	9.6**
Princeton	10	+ 0.8	127	190	182	22	1072	44	171	.1
Florida	2	− 7.3	841	888	599	13	1140	52	164	.8
Florida	2	− 8.7	431	2253	270	23	656	92	253	3.4
Florida	2	−20.5	394	16790	400	30	834	120	182	20.1**
Florida	2	−16.6	214	10837	319	18	1710	72	205	8.3**
Florida	5	− 5.5	674	1118	427	38	1334	153	153	.8
Inbred	3	+10.6	12992	4668	696	58	1547	232	164	3.1
Inbred	4	−19.2	12799	14769	276	48	1858	192	196	7.9**
Inbred	4	− 9.6	492	2815	4943	23	1071	92	128	2.6
Inbred	4	− 9.3	352	2617	257	23	1636	92	252	1.6
Inbred	4	− 6.6	24	800	216	22	1211	44	208	.7
Inbred	9	−13.7	7568	7045	325	38	1585	152	199	4.4
Inbred	9	−21.1	1504	10589	472	17	1381	68	354	7.6*
Inbred	9	−16.2	237	7893	215	23	2051	92	202	3.9
Inbred	9	−22.0	89	8756	154	22	1106	44	216	7.9*

"homozygous." In 5 instances the "homozygous" yield more than the inbreds. Three of these 5 are for one strain, Princeton 10.

Table 5 shows that 20 times out of 24 the "homozygous" laid fewer eggs than its corresponding inbred strain. Of these 20 trials 8 showed significantly lower levels of production when account is taken of the chance variations. In no instance was the "homozygous" egg yield significantly larger than the inbred. The data are consistent in showing the "homozygous" poorer in egg production than the inbreds, even though the particular "homozygous" is only a sample of the germ plasma of a highly inbred strain.

The average differences between the "homozygous" and inbred progeny is -8.3 eggs, as the "homozygous" are in general inferior producers to the inbreds. Considering each observation as equivalent, the probability that the "homozygous" are on the whole poorer producers than the inbreds is found to be beyond the 1 per cent. range by the t test. The general trend of these data confirms those of the earlier experiments.

The germ plasm within a particular race has a noticeable effect on the "homozygous" inbred differentials in egg yield. This is brought out by the comparison of the "homozygous" inbred differences in egg yields within 6 races which have repeated tests.

	d/f	Mean square
Variance between races	5	169.1
Variance within races	16	40.5

The F value is 4.2 or the probability that the races are alike is but 2 in 100.

The original races differ in their capacities to endow egg-yielding abilities to their "homozygous" equal to those of the inbred. The "Princeton" weighted "homozygous"-inbred difference in daily egg yields is -3.4; the Florida difference is -9.4; and the Inbred 92 difference is -6.3. At least two hypotheses could account for these

differences. The Princeton inbreds may be more nearly homozygous for yield genes than the other races. If this were true the "homozygous" formed from these inbreds would have less variability to draw upon and would resemble the inbred more closely than those coming from more heterozygous inbreds. Second, one race may have yield genes which perform better when homozygous than those making up the other races. Under these circumstances, the inbred and "homozygous" lines coming from the first races would be expected to have yields more closely alike than those with alleles which perform poorly when in the homozygous state.

Inbreeding behind these races would argue against the first hypothesis. The Inbred race had 97 generations of brother by sister single pair matings behind it. In theory, this amount of inbreeding should make the race nearly homozygous. But the inbred and "homozygous" lines derived from this race are more unlike than those of the more random bred Princeton race.[4] The hypothesis of differences in the ability of the alleles to perform well in the homozygous condition remains. This hypothesis is supported by many observations on such characters as fertility and general viability. There seems to be no reason to think it would not be true for metabolizing of food materials for the egg.

Discussion

There are 3 major hypotheses to account for the vigor of race crosses. One hypothesis assumes an, as yet, unexplained physiological stimulation resulting from the union of gametes of unlike origin. Another hypothesis attributes racial vigor to the union of gametes carrying different favorable dominant genes for vigor which by their dominance cover up defects which may exist in the original parental races. The last hypothesis also de-

[4] Against this reasoning, however, is the fact shown earlier that the calculation of homozygosis from only a knowledge of the system of mating employed is likely to be fallacious.

pends on genic action, assuming that the vigor of the hybrid comes from the association of unlike alleles brought in from the 2 parental races. These unlike alleles are postulated as contributing different as well as like chemical or physical stimulations favorable to the vigor of the hybrid.

The results of this study bear on these hypotheses from two sides. It is shown that the system of mating by which so-called pure races may be formed is not adequate to assure this purity. There are postulates in the mathematical model covering the genetic consequences of different systems of breeding which the functioning biological organism does not always fulfil. As such, other tests than the postulated consequences of mating systems are necessary before strict purity of races may be assumed even under the closest forms of inbreeding.

The second point has more direct bearing on these hypotheses. The contrasts in these data are between lines within races which have been bred for homozygosis by successive generations of mating within the same race, pair brother by sister and lines which have been made "homozygous" by successive generations of outcrossing to random bred tester stocks. In the first type of mating there is a tendency toward identity of both the cytoplasmic and the genic elements making up the uniting gametes. In the second type, we have identity in the genic elements of the uniting gametes. This is accomplished by outcrossing them, assuring that the cytoplasmic inheritance of the uniting gametes is different. Diagramatically, the case is as follows:

	Genes tend toward		Cytoplasms tend toward
		Inbreeding	
♂ Gametes ♀ Gametes	identity		identity
		Homozygous by outcrossing	
♂ Gametes ♀ Gametes	identity		diversity

If the physiologic stimulation hypothesis is correct, the inbred lines should have continuing low vigor and the

"homozygous" lines should be exceptionally vigorous. As the experimental results contradict this expectation, physiologic stimulation through cytoplasmic influence would seem to be ruled out.

On the other hand, the hypotheses of genic stimulation of vigor through unlike alleles would seem to hold. The inbred lines, when tested, evidently retain some heterogeneity of their alleles. The "homozygous" line should be more homozygous than the inbreds.

Under these circumstances the inbreds would be expected to have more alleles composed of different genes than those of the "homozygous." The expectations by hypotheses 2 and 3 would thus be somewhat greater vigor in the inbreds. The results support these hypotheses.

Data by Richey and Sprague (1931) bear on this view. They crossed 2 inbred lines of corn to form F_1 single crosses. The F_1 yield was 19.7 as against 3.6 for the inbred parents or the yield of the inbreds was 18 per cent. that of the race cross. Considering the parent selfed races as the zero point of vigor and the F_1 as the ultimate of yield 100 per cent., the decline in yield through successive back crosses to one of the parents was RxN F_1 100, Rx(NxR)50, Rx(NxR^2) 29, Rx(NxR^3) 22, Rx (NxR^4) 14, Rx(NxR^5) 5.6 and Rx(NxR^6) 6.2. This series bears a close relation to that which would result from the random replacement of genes from the recurrent (R) parent in the successive generations from the cross. This interpretation is further strengthened by crossing of each of these progenies, which were backcrossed to R, back to the non-recurrent inbred parent (N). The results from these crosses calculated in the same way as the R crosses[5] are N x R F_2 41, N x (NxR^2) 70, N x (NxR^3) 85, N x (NxR^4) 98, N x (Nx^5) 104, and N x (NxR^6) 97.

The expected values for the successive generations, when dominant genes are considered the cause of hybrid

[5] Fig. 10 seems to have its 2 curves calculated on different bases. In the R backcrosses the percentages are BC × – average inbred /F_1 R × N – average inbred. For the N return crosses the percentages are BCy /F_1 N × R.

vigor, are, for the R crosses, 100, 50, 25, 13, 6, 3 and 2. The expected values on this hypothesis for the N crosses are 50, 75, 88, 94, 97 and 98. The agreement between observed and expected is good. In the R series the observed tends to yield more than expected where the backcrossing has been repeated several times. In the N series the yields are less than expected 4 out of the six times, the greatest differences occurring in the first crosses to the non-recurrent parent. In both instances the differences of observed-expected are not large. The theory that dominant genes in the two inbred strains could account for heterosis is upheld by these results.

The results are not discriminatory, however, as the hypothesis of physiological stimulation arising from the uniting of unlike gametes leads to the same series as those indicated above; 100 for the cytoplasm of the F_1, 50 for the first R backcross, 25 for the second, etc.

A further attack on the problem was presented by Sprague and Tatum (1942). They attempt to separate and quantitatively assess the influence of general and specific factors for combining ability of different inbred lines. Their results indicate that either the general factors or the specific factors may be all important, or that both factors acting jointly may be responsible for hybrid vigor in particular crosses of inbred lines. The probable importance of general and specific factors is easily visualized in the hypothesis of dominance to account for hybrid vigor. General factors would be expected to be important where the physiologic stimulus hypothesis was adopted. The importance of specific factors would be less easy to fit in to this hypothesis, but it could be done if the specific effects were shown to be due to the proper relationship between the particular inbred lines which were crossed.

In the experiments presented here the individual differences are not unequivocal, but the general trend of the data points distinctly toward a genic basis rather than a physiologic stimulation as the cause of vigor in hybrids.

Summary

This paper presents a study of vigor in races, and the inbred and outcrossed "homozygous" lines which may be formed from them. Egg production in *Drosophila melanogaster* is used as the criterion of vigor.

The results show that inbreds from continued brother by sister pair matings may retain some of their genes in heterozygous condition over many generations.

"Homozygous" lines, formed by outcrossing, in general show lower yields than the inbreds from which they originated. This observation would agree with the hypothesis that the cause of hybrid vigor lies in differences in genic interaction between alleles and their reaction products. This result would not be expected, in fact, it should be in the other direction, if the cause of hybrid vigor lay in some, as yet unidentified, physiological stimulation resulting from the union of unlike gametes.

Literature Cited

Gowen, John W. and Leslie E. Johnson
1946. Am. Nat., 80: 788, 149–179.

Richey, F. D. and G. F. Sprague
1931. *Bulletin 267*, U. S. Dept. Agr.

Sprague, G. F. and L. A. Tatum
1942. *Jour. Am. Soc. Agron.*, 34: 923–932.

PRODUCTION OF FEMALE OFFSPRING BY VIRGIN FEMALES IN THE GREENHOUSE WHITEFLY, *TRIALEURODES VAPORARIORUM*, UNDER THE INFLUENCE OF HIGH TEMPERATURES[1]

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VIRGIN females of the white fly, *Trialeurodes vaporariorum*, in America have been shown to produce eggs which normally hatch into haploid males, while fertilized eggs produce diploid females (Morrill, 1903; Stoll and Shull, 1919; Schrader, 1920). It was therefore a wholly unexpected result when some virgin females subjected to high temperature produced occasional fema'es among the predominating males. Under normal temperature conditions in other experiments at the same time, virgin female white flies produced only males. The problem which immediately presented itself was to discover the manner of the production of these exceptional females. Parthenogenetic production of females by normally arrhenotokous virgin females under the influence of high temperatures seems never to have been reported. However, polyploidy has been produced in certain animals by exposing their eggs to either high or low temperatures (Just, 1929; Gross, 1932; Hasimota, 1933; Astaurov, 1936; Frolova, 1940; Griffiths, 1941; Fankhauser and Watson, 1942), and females in this case could result from a multiplication of chromosome numbers.

Parthenogenesis in the American white fly was first reported by Morrill (1903) who demonstrated that virgin females produced only males. This report was later contradicted by Hargreaves (1915) and Williams (1917), who both found that virgin females of an English race of

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T. vaporariorum gave rise only to females. Stoll and Shull (1919) confirmed through their experiments the conclusion that virgin females of the American race yielded only males and that mated females often produced many more females than males. A cytological analysis of the American race by Schrader (1920) indicated that females arose from fertilized eggs and males from unfertilized eggs; females possessed twenty-two chromosomes and males were found to have eleven.

Schrader (1926) believed that there existed two distinct races of *T. vaporariorum*. Although the two races were morphologically similar, the American race exhibited arrhenotokous, the English race thelytokous parthenogensis. This could account for the discrepancies between the conclusions of American and English workers. Thomsen (1927) described both arrhenotokous and thelytokous races from Denmark. In eggs of the thelytokous race 22 chromosomes are retained, while in the arrhenotokous race there is a reduction division, and eggs with eleven chromosomes are produced. Hence, only females were found in the thelytokous race, and both males and females in the arrhenotokous race.

Control Experiments

In order to determine whether the virgin females were of the arrhenotokous race, virgin white-fly females were placed on protected Calendula plants, and permitted to produce offspring at 20° C. The flies used in these experiments were collected on tomato plants in the University of Michigan Botanical Gardens in October, 1939.

Virgin females were collected by the following method. Leaves bearing mature pupae were picked from stock plants and placed on moist blotting paper. After two hours, the females that had emerged were collected; they were, of course, virgin. It was found that sunlight increased the rate of emergence, and by thus controlling the amount of light, virgins could be obtained at the proper time.

The control experiments were kept at room temperature which was about 20° C. Throughout the entire winter, experiments were performed beginning as soon as the weather became cool enough to permit the laboratory room to remain below 22° C. During the fall and spring months those experiments which were subjected to room temperatures above 23° C. were discarded. The virgin females were left on the plants for several weeks and removed before their offspring emerged. In forty-four experiments, a total of 410 virgin females produced 2,440 males and no females. This is in agreement with the findings of Morrill (1903), Stoll and Shull (1919) and Schrader (1920) that virgin female white flies produce only males by parthenogenesis.

High-Temperature Experiments

Virgin females were subjected to temperatures ranging from 37° C. to 45° C. for various lengths of time. This was done in an attempt to find the optimum temperature and the length of treatment needed to induce the virgin females to produce females. Both single and repeated heat shocks were employed.

Since both the insects and plants were placed at elevated temperatures, an illuminated chamber was used. This aided the plant in surviving abnormal temperatures; if the plant died while the larvae were young, all these offspring were lost. The high-temperature chamber was thirty-two inches long, twenty inches high and twenty inches deep. On each of the long sides there was an electric heater. At one end there was a double glass-plate window, outside of which were suspended two 150-watt clear-glass bulbs. Water was circulated between the glass panes to remove the heat produced by the electric bulbs.

There were twenty-five successful experiments in which virgin females were induced to produce females under the influence of high temperatures (Table 1). In these experiments, 161 virgin white flies produced 2,845 males and

TABLE 1

EXPERIMENTS IN WHICH VIRGIN FEMALES SUBJECTED TO HIGH TEMPERATURES WERE INDUCED TO PRODUCE EXCEPTIONAL FEMALES

Exp.	No. of virgin females	Temp.	No. heat treatments	Total time in hours	Offspring	
					Males	Females
		° C.				
V3h	9	38	1	5	8	1
V4h	7	39	8	8	140	24
V5h	8	38	2	28	90	16
XX2	9	38	4	19	151	32
XX5	10	38	4	18	205	10
XX6	11	38	4	18	69	2
XX7	5	38	2	28	82	1
V11X1	4	38	8	16	40	1
V14X1	7	39	4	45	48	35
Vtxh	4	40	3	36	120	1
V22X1	5	43	8	19	142	1
V51X1	9	39	2	12	253	1
V52h	9	38	3	19	145	17
V53h	7	39	2	13	186	1
V54h	7	39	2	12	114	16
V56h	6	40	1	5	75	42
V66h	3	38	4	45	199	1
V67h	2	39	5	61	75	1
V83h	6	40	1	8	9	57
V98h	7	40	2	23	0	2
V129h	8	40	1	6	179	1
V171h	4	42	4	17	225	1
V175h	5	43	3	12	148	1
V176h	7	38	2	19	124	1
V182h	6	39	3	22	18	33
Total	161				2,845	299

299 exceptional females. The temperatures ranged from 38° C. to 43° C., and the length of heat treatments varied from one five-hour treatment at 38° C. to five treatments totaling sixty-one hours at 39° C. There were four experiments in which only one high-temperature exposure was necessary to induce virgin females to produce females. The majority of the successful heat experiments involved more than two heat treatments. There was wide variation in the percentage of exceptional females in the progeny from 0.4 per cent. (253 males and one female) in V51X1 to 100 per cent. (no males and two females) in V98h.

Besides the successful attempts, there were sixty-six experiments in which virgin females were subjected to high temperatures and failed to produce exceptional females. A total of five hundred virgin females were placed at high temperatures, and produced 6,932 males and no females. There was no obvious difference between the treatment of the flies in these unsuccessful experi-

ments and that of the virgin females which did produce daughters. Indeed, in a number of pairs or groups of experiments in which the temperature and the length of treatment were identical and applied simultaneously the results were different, for in some the virgin females produced exceptional females, while in the other or others involving exactly the same conditions only males were produced. For example, experiment XX2 (Table 1) was accompanied by another which was given precisely the same treatment; the recorded parents (XX2) produced 151 males and 32 females, while in the unsuccessful one the virgin females produced 101 males and no females.

In a number of the experiments, the virgin females were permitted to lay eggs at room temperature, before they were transferred to another plant and heat shocked. This was done to test the females before they were subjected to high temperatures. In one experiment ten virgin females were allowed to lay eggs for several days. The virgin females were then removed and placed on V14X1 which was subjected to four heat treatments at 39° C. for a total of 45 hours (Table 1). Three males and no females were produced before treatment, but forty-eight males and thirty-five females after treatment. In another experiment twelve virgin females produced (in five days) sixty males and no females; the virgins were then transferred to V22X1 (Table 1) and heat treated a total of nineteen hours at 43° C., after which they produced 142 males and one female. These groups of families present some evidence that the virgin females were male-producing before being subjected to high temperatures.

Pupae and Eggs Subjected to High Temperatures

After adult virgin females had been induced to produce females under the influence of high temperatures, the pupae and eggs were then subjected to the same treatment to determine whether or not exposure of these

stages to the high temperatures would influence the adult female. Pupae were treated at temperatures varying from 38° C. to 47° C. The virgin females which emerged from these pupae were then placed on protected plants and the offspring examined for exceptional females. None were found in nine experiments in which a total of sixty-seven virgin females produced 618 males and no females.

Eggs which were laid by virgin females were exposed to temperatures of 38° C., 39° C. and 40° C. with the hope of producing exceptional females. In these experiments 124 males and no females were produced.

From this evidence it appears that high temperatures applied to the pupa do not cause it, as a virgin adult, to produce females; nor does exposure of unfertilized eggs to high temperatures induce them to develop into females.

Virgin Females Subjected to Low Temperatures

An attempt was made to duplicate the heat-shock results with low temperatures. Virgin females were placed on plants and kept at room temperature for a day to feed and obtain strength to endure the cold. They were then placed at low temperatures and later tested for thelytoky. The low temperature chamber was cooled by brine and maintained temperatures ranging from 4° C. to 7° C. In six experiments a total of forty-two virgins were subjected to low temperatures for 18 to 98 hours and produced 650 males and no females. The low temperatures did not induce the virgin females to produce females.

Virgin Females Subjected to Both Low and High Temperatures

Virgin females were first exposed at low temperatures and then transferred to high temperatures with the hope that the sudden change and the greater range of temperature would induce the virgin females to produce some exceptional females. In the majority of the experiments

the virgin females were placed at temperatures near 5° C. for from 11 to 33 hours, and then removed from the cold chamber and placed at temperatures about 40° C. In four of these experiments no females were produced, but in the fifth one, V71CH, one or more of the ten virgin females were induced to produce females (Table 2). In this latter

TABLE 2

VIRGIN FEMALES SUBJECTED TO LOW TEMPERATURES FOLLOWED BY HIGH TEMPERATURES

Exp.	Virgin parents	Temp.	No. of treatments	Total time	Temp.	No.	Total time	Offspring	
								Males	Females
		° C.		*hrs.*	*° C.*		*hrs.*		
V32X1	13	4	3	33	40	3	68	124	0
V34X1	7	5	1	12	40	1	13	24	0
V45X1	6	5	1	11	40	1	12	178	0
V46X1	10	4	1	12	38	1	12	247	0
V71CH	10	6	3	87	40	3	38	80	16
Total	46							653	16

experiment the flies were left at 6° C. for 87 hours. This may be the reason why the virgin females were induced to produce females. However, as shown above, high temperatures alone sometimes induce the virgin females to produce females, and thus the low temperature may have had no influence.

IS THE HIGH-TEMPERATURE EFFECT TRANSMITTED?

Virgin females were obtained from exceptional females and were first tested for thelytoky at room temperature. These virgins were later exposed to high temperatures. In one series of experiments, five virgin females were obtained from progeny produced by the exceptional female in V11X1 (Table 1). At room temperature these virgins produced 116 males and no females. Four of the virgin females (one lost on transfer) were then subjected to high temperatures and produced 120 males and one female (Vtxh, Table 1).

Out of the thirty-five exceptional females produced by the influence of high temperatures in V14X1 (Table 1), four were known to be virgins and were tested by being

isolated on a plant; these virgins produced 123 males and no females. From the other thirty-one females that had mated with their brothers, virgin females were obtained. These virgin females produced only males at room temperature, and after being exposed to high temperatures continued to produce males.

From the above results it appears that exceptional females and their progeny behave like stock females with regard to parthenogenesis. Virgin females produced only males at room temperature, and when subjected to high temperatures the virgin females sometimes produced females along with the males. Thus, one can conclude that the high temperature effect, in which the virgin female is induced to produce females, is not transmitted from one generation to another.

Colchicum Experiments

Another method of inducing polyploidy has been the influence of the alkaloid colchicine on chromosome separation (Blakeslee and Avery, 1937; Nebel and Ruttle, 1938). Since colchicine is water-soluble, the drug could not penetrate the wax-covered body wall if the insects were to be dipped in a solution of colchicine. Microinjection apparatus was not available, so a crude method was employed. The virgin females were placed on *Colchicum autumnale*, the plant from which colchicine is extracted, and allowed to feed. Although colchicine is extracted from the seeds of the plant, there is enough present in the leaves to poison cattle (Hegi, 1926). In feeding upon the plant sap, colchicine was probably taken into the alimentary canal, and there was a possibility that some of the colchicine could diffuse and affect the eggs.

The virgin females were left on the Colchicum plant for periods ranging from two to twenty-four hours, and removed to young Calendula plants. Although the flies were observed to go through the feeding motions, most of the females appeared to be starved after twenty-four hours. The Colchicum plant had hard, thick leaves (like

those of the rubber plant) and this may have prevented the stylet from reaching the leaf sap. The Colchicum plants were grown under large lantern globes to prevent stray white flies from getting on the plant before the virgin females were placed on them. The insects were placed on young leaves, for these leaves were softer and the flies would have less difficulty in feeding.

Although twenty-one experiments were started, only in eleven were offspring produced. Exceptional females were found in two of these. In experiment C1 two exceptional females were produced, and one female was counted in C6 (Table 3). In all, a total of sixty-six virgin

TABLE 3

VIRGIN FEMALES ALLOWED TO FEED ON *Colchicum autumnale* AND THEN TRANSFERRED TO A CALENDULA PLANT

Exp.	Virgin females used as parents	Length of treatment	Offspring	
			Males	Females
		hrs.		
C1	10	24	18	2
C2	3	20	1	0
C3	6	18	43	0
C4	2	22	7	0
C5	5	24	115	0
C6	5	24	100	1
C7	10	24	61	0
C9	6	22	141	0
C11	5	23	46	0
C16	4	24	37	0
C21	10	24	64	0
Total	66		633	3

females produced 633 males and three females (Table 3). The exceptional females appeared to be similar to the stock (control) females.

CYTOLOGY

Since it was suspected that the production of females by virgin females was the result of polyploidy, chromosome counts were made of adults, pupae, larvae and eggs of both control and heat-produced females. The whitefly material was first dipped into Carnoy's No. 2 solution to remove the wax and then fixed in Navashin's fluid. Heidenhain's iron hematoxylin was used as the stain.

The chromosome number of all the stock females

counted was twenty-two. The chromosome number of five exceptional females was also determined and there was no difference. The control and exceptional pupae were found to have eleven chromosomes in the males and twenty-two in the females. All the stock embryos examined for chromosome number had either eleven or twenty-two chromosomes. At the metaphase of the first division in the maturation of both a control egg and an egg produced by an exceptional female, there were eleven tetrads. The eggs at the end of the second division of the maturation process possessed eleven chromosomes, which is in agreement with the work of Schrader (1920) and Thomsen (1927) on white-fly cytology.

Discussion

The production of exceptional females by subjecting the virgin females to high temperatures can best be explained by assuming that the maturation process is altered and the diploid number of chromosomes is retained by the egg. Under normal conditions the virgin white fly produces only males, and mated females produce both males and females; a fertilized egg results in a female. The cytological examination demonstrated that the males have eleven chromosomes while the females possess twenty-two. An egg laid by a virgin female has eleven chromosomes at the end of the maturation division and is destined to become a male. However, under the influence of high temperatures the virgin females are induced to produce some exceptional females. This may be due to the retention of the diploid number of chromosomes, for all the chromosome counts of the exceptional females indicated the presence of twenty-two chromosomes.

In the silkworm studies (Astaurov, 1936; Frolova, 1940) and the newt experiments (Griffiths, 1941; Fankhauser and Watson, 1942) in which polyploidy was induced in eggs by extremes of temperature, the eggs were outside the body of the female. The eggs were in some

phase of the maturation process when the low or high temperature was applied. In the case of the white flies the eggs were subjected to the high temperature while still in the female. In the multiple-heat-shock series, it was possible that some of the eggs were in one of the maturation divisions when placed in the high-temperature chamber. This can account for the experiments in which only one or two exceptional females were produced. However, one can not use the theory of direct action of high temperatures on the maturation divisions in the case of experiment V83h (Table 1), in which a short heat treatment induced the virgin females to produce a comparatively large number of females (57 females and nine males). The white-fly female lays about three eggs per day. In experiment V83h the six virgins that were used as parents were subjected to 40° C. for eight hours. The maximum number of eggs that were in the process of maturation during the period of high temperature must have been about twenty. However, fifty-seven females were produced. One may assume that the high temperature affected the immature eggs in the ovaries of the virgin females. These eggs, when they have matured to the extent necessary to start the maturation process, may still retain the heat influence which results in the eggs retaining the diploid number of chromosomes.

The production of the exceptional females by virgin females occurred only in the experiments in which adult flies were exposed to high or low temperatures. The experiments in which pupae were heat-shocked and the virgin females emerging from them tested for thelytoky were few in number. However, in the nine experiments not one female was found among the 618 males produced. There were three experiments in which unfertilized eggs were subjected to high temperatures and failed to produce exceptional females. It would appear that the heat effect upon the eggs or germinal tissue of the females occurs only in the adult female.

The multiple heat shocks appeared to induce the virgin

females to produce females with greater ease than single-shock experiments, for in the successful experiments there were four single and twenty-one multiple-heat-shock experiments (84 per cent. multiple), while in the unsuccessful experiments there were twenty-one single and forty-five multiple (68.1 per cent. multiple). It would seem that several heat shocks in succession would be more likely to catch the virgin females in the proper stage for the heat to affect the eggs.

The existence of a thelytokous race of *T. vaporariorum* in Denmark has been reported by Thomsen (1927). Since there were many generations in which no male appeared, there must be some genetic control which maintains the diploid condition. The action of the high temperature may be the altering of a process or processes which result in the egg maintaining its diploid condition. The temperature change may have the same effect as a gene or genes in this case. The genetic processes in control of the maturation may be altered by the high temperatures, but the effect is not passed on to future generations, for this heat influence upon virgin females is not permanent or a fixed mutation, because the exceptional females and their progeny when tested behaved like normal white flies. Even the exceptional females when obtained as virgins and tested produced only males.

If a mutation had been discovered, it would have aided in determining exactly where in the maturation process the disruption takes place that results in the egg possessing a diploid number of chromosomes. One of the divisions may be suppressed, or the fusion of one of the polar bodies with the egg nucleus can produce a diploid egg. Frolova (1940) reported, in the study of the maturation of unfertilized eggs of *Bombyx mori* induced to develop by high temperatures, that the reduction division was eliminated, one polar body was formed, and the diploid chromosome number was retained in the pronucleus. This inhibition of the reduction division was due to the stretching of the spindle, which prevented the

rotation of the tetrads and the formation of an elimination plate.

The Colchicum experiments were not as successful as the high-temperature experiments in inducing virgin females to produce females. The three exceptional females produced by virgin females after feeding on the Colchicum plant were morphologically similar to the control females. These exceptional females were probably produced by the colchicine diffusing through the alimentary canal and affecting the eggs by means of the open circulatory system. Colchicine is well known for its ability to induce polyploidy by disrupting chromosome separation in animals and plants. Hence, these exceptional females add more evidence that females produced by virgins were due to polyploidy which probably resulted from faulty chromosome separation.

In this study of the influence of high temperature upon the production of females by virgin females, the problem of sex determination is encountered. As has been mentioned above, the would-be males are induced to become females under the influence of high temperature by a probable retention in the egg of the diploid number. From the results, not much can be said about the sex determination of this particular insect other than what has been written by other authors. No sex chromosomes can be distinguished. In the white fly, possession of a haploid or diploid number of chromosome is the feature which determines sex. Thus a diploid number of genes acts differently from a haploid set of genes in production of sex in this insect. It is evident that in doubling the chromosome number the processes that would have gone on to produce a male white fly are altered and a female results. The high temperature to which the virgin females were subjected does not take the place of an extra set of genes, but produces an influence on the maturation division, which results in the egg retaining its diploid condition.

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SUMMARY

Under normal temperature conditions (20° C.), the virgin white-fly female produces only males; females result from fertilized eggs. Virgin white-fly females, when subjected to temperatures about 38° C., were induced sometimes to produce exceptional females.

In forty-four control experiments, 410 virgin females produced 2,440 males and no females.

In a total of ninety-one experiments 661 virgin females were subjected to temperatures ranging from 37° C. to 45° C. In twenty-five of the experiments, 161 virgin females produced 299 exceptional females and 2,845 males. The other sixty-six experiments failed to produce females. Multiple heat treatments were more successful in production of exceptional females.

Low temperatures failed to induce virgin females to become thelytokous. Virgin females that had been exposed to high temperatures while in the pupal stage failed to produce females. Unfertilized eggs exposed to high temperatures developed only into males.

Exceptional females have the same diploid number of chromosomes, twenty-two, as the stock (control) females. Males have eleven chromosomes. Virgin exceptional females produced only males at room temperature; the heat effect is not passed on from generation to generation.

Virgin females were permitted to feed on *Colchicum autumnale*, which contains colchicine, and then tested for thelytoky. In two of the eleven experiments, three exceptional females were found.

The production of females by virgin females may be due to the retention of the diploid number of chromosomes in the egg under the influence of high temperature. The second polar body may fuse with the egg nucleus or

there could be a faulty separation of the chromosomes during one of the divisions in the maturation process.

LITERATURE CITED

Astaurov, B.
1936. *Compt. Rend. Acad. Sci. l'URSS.*, 2: 283–6.
Blakeslee, A. F., and A. G. Avery
1937. *Jour. Hered.*, 28: 393–411.
Fankhauser, G., and R. C. Watson
1942. *Proc. Nat. Acad. Sci.*, 28: 436–44.
Frolova, S. L.
1940. *Compt. Rend. Acad. Sci. l'URSS.*, 27: 601–3.
Griffiths, R. B.
1941. *Genet.*, 26: 69–88.
Gross, F.
1932. *Naturwissen.*, 20: 962–7.
Hargreaves, E.
1915. *Ann. Appl. Biol.*, 1: 303–34.
Hasimota, H.
1933. *Bull. Imp. Seri. Exp. Sta.*, 8: 359–81.
Hegi, G.
1926. "Illustrierte Flora von Mittel Europa." Munich. Lehmanns. Pp. 195–8.
Just, E. E.
1929. *Biol. Bull.*, 57: 439–42.
Morrill, A. W.
1903. *Mass. Agr. Tech. Bull. Exp. Sta.*, 1: 1–65.
Nebel, B. R., and M. L. Ruttle
1938. *Jour. Hered.*, 29: 3–9.
Schrader, F.
1920. *Jour. Morph.*, 34: 267–98.
Stoll, N. R., and A. F. Shull
1919. *Genet.*, 4: 251–60.
Thomsen, M.
1927. *Zeit. Wissen. Biol. Abt. B. Zell. Mikr. Anat.*, 5: 1–116.
Williams, C. B.
1917. *Jour. Genet.*, 6: 255–67.

STABILITY OF THE SEX ALLELES OF HABROBRACON

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In the parasitic wasp *Habrobracon juglandis* (Ashmead) sex determination has been shown (Whiting, 1943) to depend upon a single series of allelic factors (xa, xb, xc, etc.) with any haploid or homozygous diploid, male; any heterozygous combination, female. Fraternities from mated females are of two types—two-allele in which the father (haploid) has one of the alleles present in the mother (xa/xb ♀ × xa ♂, for example) and three-allele in which the father possesses a third allele not present in the mother (xa/xb ♀ × xc ♂).

Both types of fraternities include haploid males (xa and xb) from unfertilized eggs. Three-allele fraternities have two types of females (xa/xc and xb/xc) phenotypically indistinguishable. Two-allele fraternities include diploid males (xa/xa) as well as females (xa/xb). Diploid males are of relatively low viability and therefore not numerically equal to their sisters. In summary, two-allele fraternities tend to differ from three-allele (1) by having diploid males, (2) by having lower female ratio (females/haploid males) and (3) by being smaller.

If a homozygous recessive orange-eyed female is mated to a wild-type black-eyed male, the diploid sons may be separated from their haploid brothers by the paternal black eye color.

The existence of a large number of sex alleles suggests the possibility of mutation from one to another. This might, however, remain unnoticed in any one stock, xa/xb, for if xb mutated to xc and the latter became established, replacing one of the original alleles, the mutated stock, xa/xc or xb/xc, would behave in closecrosses exactly as before. If, however, a single two-allele stock were di-

vided into two stocks which were bred separately for several generations, subsequent crosses would reveal mutation by producing three-allele fraternities.

Bostian (1934) graded orange eye color to wild-type stock 11-+ and thus derived orange stock 11-o with the same sex alleles as 11-+. Subsequently Anderson (1935) carried out experiments involving 307 pairings of stock 11-o females by stock 11-+ males. Only eleven fraternities lacked diploid males and of these only one was large enough to be significantly different from the others. The female ratio for the two-allele fraternities was (8902 ♀♀ to 7501 haploid ♂♂) 1.187, but in this "mutant" fraternity it was (83/21) 3.952. Since ratio of diploid males to females in general was (2967/8902) 0.333, about twenty-eight diploid males should have occurred had this fraternity not been "mutant." Many other crosses of stock 11-o by stock 11-+ have been made, but only the smaller fraternities have lacked diploid males as an error of sampling. Mutation of the sex alleles in this material must, therefore, be very infrequent if it occurs at all. The single "mutant" fraternity of Anderson may have resulted from some change in the material other than mutation in a sex allele.

Two stocks of "Lancaster" material having the same sex alleles before 1925 were found in 1929 to have only one allele in common (Whiting, 1940). Mutation may have occurred unless one of the stocks had been contaminated. The published data indicate that the sex alleles are not frequently mutable even if it be granted that the two cases cited are mutations.

Of the three types of wasps commonly produced, the females (diploid) are the most stable and viable. Haploid males are normally almost as viable as females but are affected by recessive lethals and semilethals if their mothers happen to carry them. Diploid males have very low viability. Measurements of wing cell size have been made by the Dobzhansky method of wing-microchaetal counts. Cells of haploid males tend to be but slightly

smaller than those of females, in some stocks being actually larger, while diploid males have abnormally large cells differing in different stocks but in general greatly exceeding those of females. It has been suggested that this condition is to be expected because, in the evolution of the Hymenoptera, haploid males have acquired cell size greater than that found in other haploids and diploid males have the same genic balance as haploid males but with doubled chromatin content. Females, although diploid, will not be thus affected if it be supposed that "genes" for increased cell size are recessive and are contained within the sex-determining x element. Many of these "genes" will therefore be heterozygous in females but all will be homozygous in diploid males. Evidence indicates that differences between stocks in mortality of diploid males are associated with differences in cell size.

Since in two-allele crosses female-producing and (diploid) male-producing zygotes occur with equal frequency, ratio of diploid males to females is a good measure of relative viability. Under standard laboratory conditions about two thirds of the eggs of mated females are fertilized until such females exhaust their supply of sperm. Approximately equal numbers of females and of haploid males are therefore to be expected from two-allele crosses, but in three-allele fraternities females should be twice as frequent as their (haploid) brothers. These ratios involving haploid males are subject to fluctuations due not only to varying mortality of the different sex types but also to conditions affecting proportions of eggs fertilized. In analysis of data average numbers of females or of haploid males produced per day or per fraternity may be used in addition to female ratio (females/haploid males).

Previous to formulation of the multiple allele hypothesis it was thought that multiple independently segregating factors might govern sex, either directly or by controlling some process of differential maturation. Various

experiments were planned and data collected in attempts to analyze the factors involved. Most of these data have been reviewed in the light of the multiple allele theory (Whiting, 1940).

Conditions of the Experiment

Results from an experiment, suggested by P. W. Whiting and carried out by C. H. Bostian, begun in March, 1936, and continued until 1941, have not been published. Although the purpose, and therefore the plan of this experiment, were different from one that might be undertaken at the present time in the light of our knowledge of multiple sex alleles, the data are of some interest since they show that in a two-allele population strains with widely different ratios of the three sex types may be isolated by inbreeding and since they suggest that the explanation of "mutant" fraternities may be other than mutation of a sex allele.

The parents (P) in the experiment were a wild-type female of Fresno, California, stock (sex alleles xe/xf) and a male of unrelated orange stock 11-o (sex alleles, xc/xd). A single daughter (F_1), +/o and xc/xe for example, was first bred unmated and then crossed with one of her own orange sons (xc or xe). All material in the experiment is derived from this single female and hence only two sex alleles are expected unless mutation occurs in one of them.

From the fraternity (F_2) resulting from the backcross of this female to her orange son, twelve pair matings were made of wild-type females by orange brothers. $+/o \times o$, (F_2), and sibling matings were continued for eight further generations (F_3–F_{10}), both orange and black eye colors being retained in each line. From the progeny (F_{11}) of the final pairings orange sibling pairs and black sibling pairs were set (March, 1937) and orange and black stocks were derived from each pair. These orange and black sister stocks were given the same numerals, 1o and 1+ being derived from one F_{10} pair, 2o and 2+ from another, etc.

Between 1937 and 1941 crosses were made between orange females and black males of these stocks. Since only two sex alleles should be present in the entire population, all crosses should produce two-allele fraternities. Parents from stocks having the same numerals should be of very similar genotype. Heterozygosis is reduced 50 per cent. with every generation of sibling matings in Habrobracon because of male haploidy. Any F_{11} fraternity should therefore have only 1/1024 as many genic differences as were present in the F_1 female. Diploid offspring from crosses of stocks with the same numeral should be relatively homozygous in contrast to those whose parents were inbred along different lines. As regards the latter, varying amounts of heterozygosis should occur according as chance may have isolated diverse factors segregating in oogenesis of the F_1 female and may have retained them in later generations of inbreeding.

In the present paper the term closecross is used to designate crosses betweeen "sister" stocks, orange and black-eyed, with the same numeral while outcross designates crosses of orange females to black-eyed males bearing a different numeral. The term outcross was at first used to designate crosses between stocks of different wild ancestry. Since these were later found to bear different sex alleles, outcross has often indicated a three-allele cross. As stated above, only two known sex-alleles are involved in the experiment reported here.

Summaries according to Lines Used in Crosses

Because of their low fecundity and viability some of the stocks were lost before they were used in crosses. Table 1 records the data from the types of crosses that were made. These are arranged according to increasing viability of diploid males relative to their sisters (+♂♂/+♀♀) showing a range from less than 1 per cent. to over 24 per cent. There appears to be no marked association of female ratio or of average number of

TABLE 1

CROSSES ARRANGED ACCORDING TO INCREASING VIABILITY OF DIPLOID MALES RELATIVE TO FEMALES (4TH COLUMN, +♂/+♀). FEMALE RATIO IS EXPRESSED AS RATIO OF FEMALES TO HAPLOID MALES (5TH COLUMN, +♀/o♂). FECUNDITY IS GIVEN IN RESPECT TO FEMALES PER FRATERNITY (6TH COLUMN, +♀/FRATERNITIES) AND TO HAPLOID MALES PER FRATERNITY (7TH COLUMN, o♂/FRATERNITIES).

Parental lines Orange ♀♀ by wild type ♂♂	Offspring Fraternities	♀♀	+♂/+♀	+♀/o♂	+♀/fraternities	o♂/fraternities
6o × *5+*	43	1484	.0067	1.62	34.51	21.30
6o × *3+*	51	2271	.010	1.11	44.53	40.20
6o × *2+*	47	2204	.023	1.20	46.89	39.00
5o × *3+*	53	2021	.023	1.14	38.13	33.34
5o × *5+*	40	1587	.059	1.17	39.67	33.97
5o × *10+*	56	1888	.064	1.12	33.71	30.03
6o × *9+*	43	1370	.075	1.77	31.86	17.95
9o × *5+*	78	2312	.089	1.44	29.64	20.64
5o × *2+*	34	1315	.090	1.32	38.68	27.85
9o × *2+*	46	1871	.097	1.12	40.67	36.39
5o × *9+*	37	2107	.101	1.26	56.95	45.27
8o × *8+*	39	1539	.110	1.32	39.46	29.92
1o × *1+*	28	451	.113	1.21	16.11	13.32
9o × *9+*	58	2642	.128	1.71	45.55	38.97
5o × *8+*	42	2468	.130	1.41	58.76	41.69
2o × *5+*	44	1775	.150	1.32	40.34	30.45
10o × *2+*	45	2090	.153	1.59	46.44	29.13
7o × *7+*	47	1491	.156	1.21	31.72	26.23
3o × *3+*	43	1135	.159	1.29	26.40	20.49
2o × *2+*	50	2360	.160	1.28	47.20	36.76
2o × *1+*	53	2665	.179	1.77	50.28	28.42
2o × *3+*	50	2264	.210	1.35	45.28	33.54
2o × *9+*	46	2197	.218	1.47	47.76	32.65
10o × *10+*	62	2478	.230	1.43	39.97	27.97
2o × *10+*	64	3805	.232	1.40	59.45	42.36
2o × *7+*	37	1642	.242	1.38	44.38	32.03
2o × *8+*	42	1611	.242	1.75	38.36	21.83

females or of haploid males per fraternity with viability of diploid males relative to females. A combination of especially low fecundity is 1o by 1+ with intermediate diploid male viability. High fecundity is shown by 5o by 9+ and by 5o by 8+ with intermediate diploid male viability and by 2o by 10+ with high.

There were eight types of closecrosses. No closecrosses were made with 6o females, but the low position of their outcrosses indicates factors for low viability of diploid males in this stock. That lines 5o and 5+ likewise have such factors for low viability is indicated by the outcrosses as well as by the closecrosses. Six of the seven types of outcrosses made with 2o females have higher diploid male viability than any other outcrosses while the four outcrosses with 2+ males are of relatively low rank. 2o by 5+ is of higher rank than 5o by 2+, 2o by 9+ is higher than 9o by 2+ and 2o by 10+ is higher than 10o by 2+. These facts suggest an influence of maternal genotype exceeding that of paternal genotype

on diploid male viability, a possibility which should be further investigated.

Although the ranks of the outcrosses 5o by 3+, 9o by 2+ and 10o by 2+ are each inferior to those of closecrosses bearing the numerals 2, 3, 5, 9 and 10, the viability of the diploid males from 2o by 8+, 2o by 7+, 2o by 10+, 2o by 9+, 2o by 3+ and 5o by 8+ is increased over that of the closecrosses. All outcrosses involving 2o females are of higher rank than the closecrosses 2o by 2+ except 2o by 5+, the 5+ males apparently determining very low diploid male viability. These facts suggest that heterosis may increase viability of diploid males.

Summaries according to the Presence of Diploid Males

The data of the experiment were likewise summarized according to the presence or absence of diploid males in the individual fraternities.

Among the total of 367 closecross fraternities, 342 included diploid males while twenty-five lacked them. Twenty-three of the latter were small or had relatively few females (+♀ 369, o♂ 314, +♀/o♂ 1.175, +♀/fraternities 16.04, o♂/fraternities 13.65). In contrast to these the 342 fraternities with diploid males (+♀ 13130, +♂ 1987, o♂ 10472, +♂/+♀ 0.151) had higher female ratio (1.25) and averaged more females (38.39) and more haploid males (30.62) per fraternity. It is then probable that these twenty-three fraternities without diploid males lacked them merely through error of sampling. The remaining two fraternities without diploid males, one from 8o by 8+ (+♀ 75, o♂ 21) and one from 10o by 10+ (+♀ 109, o♂ 38) have higher female ratio, 3.12, and more females per fraternity, 92.00, than the average of the others from these two types of crosses, while the number of haploid males (29.50) is not increased. These facts have suggested mutation in a sex allele and therefore these two fraternities may tentatively be called "mutant."

The outcrosses of 2o, 5o, 9o and 10o females produced

680 fraternities with diploid males (+♀ 30620, +♂ 4883, o♂ 22329, +♀/o♂ 1.37, +♀/fraternities 45.03, o♂/fraternities 32.84, +♂/+♀ 0.159). Forty-two outcross fraternities from these females (+♀ 961, o♂ 780, +♀/o♂ 1.23, +♀/fraternities 22.88, o♂/fraternities 18.57) lacked diploid males, probably as an error of sampling, but five (one from 2o by 1+ and four from 2o by 10+) totalling 452 wild-type females and 149 orange males, had high female ratio, 3.03, and many females per fraternity, 90.50, without increase in haploid males (29.80), suggesting mutation in a sex allele.

In all the groups of outcrosses of 6o females the viability ratio for diploid males was very low and consequently many fraternities lacked them altogether. There were ninety-two with diploid males (+♀ 3817, +♂ 186, o♂ 2847, +♀/o♂ 1.34, +♀/fraternities 41.48, +♂/+♀ 0.049, o♂/fraternities 30.95) and ninety-two without (+♀ 3512, o♂ 2724, +♀/o♂ 1.29, +♀/fraternities 38.17, o♂/fraternities 29.61). The female ratio is lower and females are fewer on the average in the fraternities without diploid males than in those with them and this proved true for most of the groups. In one group of crosses with 5+ males, however, the reverse condition obtained. These (32) were made earlier than the others (11) with 5+ males. There were five with diploid males (+♀ 172, +♂ 7, o♂ 155, +♀/o♂ 1.11, +♀/fraternities 34.40, o♂/fraternities 31.00, +♂/+♀ 0.041) and 27 without (+♀ 1067, o♂ 532, +♀/o♂ 2.01, +♀/fraternities 39.52, o♂/fraternities 19.70). The high female ratio of the latter might suggest mutation in a sex allele. However, average numbers of females per fraternity are only slightly increased instead of being nearly doubled as would be expected if this group were composed entirely of three-allele fraternities. If the group of twenty-seven included some two-allele fraternities lacking diploid males as an error of sampling, its excess over the five with diploid males might be explained. The factor of larval crowding with differential mortality of the larger three-allele fraternities would explain the failure of females per fraternity to be mark-

edly increased and this would also account for the greatly decreased number of haploid males. These facts may likewise be explained without resort to a third sex allele by assuming the presence of multiple factors physiologically unfavorable to development of males and favoring development of females. The five fraternities with diploid males would then represent a fluctuation with fewer females and more males, both haploid and diploid. A third possibility is presence of a semilethal which, if sex-linked, would tend to maintain its heterozygosis in the females.

Selection Test

A selection test was made from one of the "mutant" fraternities (F_1) (from 2o female by 10+ male), which contained 110 wild type females and 33 haploid males. (Diploid males were not recorded in the even-numbered generations, F_2, F_4, F_6, in this selection test because half of their haploid brothers were wild type and they were, therefore, not readily separable.) A fraternity of the "mutant" type appeared in F_3 (+♀ 76, o♂ 29) and three occurred in F_5 (+♀ 103, o♂ 33; +♀ 70, o♂ 18; +♀ 91, o♂ 32), but in F_7 all fraternities yielded diploid males. Total number of individuals counted in this selection test include sixty-four fraternities with diploid males (+♀ 3168, +♂ 415, o♂ 1916, +♀/o♂ 1.65, +♀/fraternities 49.50, o♂/fraternities 29.94, +♂/+♀ 0.131) and fourteen without (+♀ 700, o♂ 309, +♀/o♂ 2.27, +♀/fraternities 50.00, o♂/fraternities 22.07). Higher female ratio is here associated with lack of diploid males. Average numbers of females per fraternity are essentially similar in the two groups, the difference in female ratio being due to fewer haploid males in the fraternities lacking diploid males. The explanation of conditions here may be similar to one of those suggested for the outcrosses.

Suggested Explanation of "Mutant" Fraternities

The eleven so-called "mutant" fraternities of the

present experiment, as well as the two reported previously, may differ from those containing diploid males, not because of mutation to a third sex allele, but for other reasons. In that case it might be possible to select fraternities including diploid males but showing the other characteristics of three-allele fraternities. Ten large fraternities with high female ratios were thus selected from among the closecrosses and fifty-one from among the outcrosses. As examples with many diploid males may be given three from the closecrosses:—+♀ 72, +♂ 18, o♂ 32 from 5o by 5+; +♀ 67, +♂ 12, o♂ 26 and +♀ 53, +♂ 27, o♂ 29 from 10o by 10+; and three from the outcrosses:—+♀ 100, +♂ 28, o♂ 28 and +♀ 84, +♂ 12, o♂ 19 from 2o by 1+ and +♀ 72, +♂ 10, o♂ 20 from 2o by 8+. Factors favoring high fecundity in general but with recessive semilethals reducing number of haploid males may be suggested to account for these. There is also to be considered chance of high ratio of eggs fertilized, increasing the two zygotic types at expense of the haploid males.

A few of these selected fraternities had very small numbers of diploid males, such as:—+♀ 77, +♂ 1, o♂ 31 from 5o by 5+ and +♀ 82, +♂ 3, o♂ 16 from 5o by 9+. Sex-linked recessive semilethals or non-sex-linked factors unfavorable to males in general may be the explanation. From such material it is evident that errors of sampling may produce the "mutant" fraternities without diploid males.

General Discussion

Whether the principles of sex-determination which have been demonstrated for Habrobracon apply to Hymenoptera in general and to other groups with haploid males is as yet an unsettled question. The problem has been recently reviewed in detail (Whiting, 1945). Two principles have been shown:—the basic one of complementary determination of females (Whiting, 1935) and the supplementary one of multiple allelism of the factors involved (Whiting, 1943). The former may be of general

application for species with male haploidy, while, instead of the latter, a single pair of complementary sex factors or several independently segregating pairs may be found to function in other species.

It can not, of course, be asserted that even in Habrobracon we may not at some time turn up a second sex-differentiating "locus." It has been pointed out that in such a case sex-linkage relationships should be different from those demonstrated in our tested stocks. However, this would not necessarily indicate a sex factor different in its original nature, za/zb. A second prime type might be found with the x series differently placed in the linkage map. One may speculate about the possibility of deriving homozygous (xa/xa xb/xb) or even haploid (xa xb) females from the hybrids, but inviable types would be expected. Chromosomal rearrangements involving x may well have been a primary factor for species isolation in groups with male haploidy.

Whatever may be the true explanation for the "mutant" fraternities discussed in this paper, there is, as yet, no need for postulating mutation or translocation of x or the presence of a second sex differentiating series, z, of different genic origin. The ratios of the three sex types may, as already stated, be tentatively explained by differences affecting viability and percentages of eggs fertilized.

Unfortunately for ease of genetic analysis, linkage in Habrobracon is exceedingly loose and lack of giant salivary chromosomes prevents the convenient cytological approach available in the Diptera. Discovery of members of the series of x-alleles beyond the nine already demonstrated may be expected. Tests for mutation of x within a two-allele population, marked against all chance of contamination by a combination of mutant genes, may be carried out and search for a different prime type may be undertaken. Such work with *Habrobracon juglandis*, as well as genetic studies on other species with haploid males, would be of great interest from the point of view of evolution and of the nature of sex genes.

SUMMARY

A single female heterozygous for orange eye-color and derived from a cross of unrelated parents was bred to one of its own orange sons. Descendants were inbred by sibling matings in different lines for ten generations with orange and wild type black eye-color retained in each line. Orange and black stocks were then derived and the orange females were crossed in various combinations with the black males. Total progeny counted were 56,913 females, 7,471 diploid males and 41,899 haploid males. Only two members of the single series of multiple sex alleles are to be expected because the entire population was derived from a single female.

The data suggest an influence of maternal genotype exceeding that of paternal on viability of diploid males and also an increase in their viability due to heterosis.

Diploid male viability as compared with that of females showed no association with sex ratio or with average numbers of haploid males or of females per fraternity.

There were, however, eleven so-called "mutant" fraternities, suggesting possible mutation of a sex allele since they lacked diploid males and had high female ratios and large numbers of females. A genetic basis is suggested but their deviation may be explained by conditions causing high fecundity and high ratios of eggs fertilized, by the presence of a sex-linked semilethal and by errors of sampling. There is therefore as yet no conclusive evidence for mutation of the sex alleles of Habrobracon.

LITERATURE CITED

Anderson, R. L.
1935. AM. NAT., 69: 183–187.
Bostian, C. H.
1934. *Biol. Bull.*, 66: 166–181.
Whiting, P. W.
1935. *Jour. Hered.*, 26: 263–278.
1940. *Jour. Morph.*, 66: 323–355.
1943. *Genetics*, 28: 365–382.
1945. *Quart. Rev.*, 20: 231–260.

THE "VIROID" THEORY IN RELATION TO PLASMAGENES, VIRUSES, CANCER AND PLASTIDS

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IT has long been known that cockroaches harbor intracellular "bacteroids" in their fat bodies. These are not parasites. They are necessary for the continued existence of their hosts, for if the bacteroids are killed by means of penicillin, their hosts die, as shown recently by C. T. Brues and Ruth C. Dunn (1945). The penicillin itself is non-toxic to the host tissues proper. The bacteroids are therefore symbionts. They undoubtedly are necessary for some cell process, perhaps for the building up of some vitamin, for its utilization, or for some other metabolic process.

Bacteroids are found in the fat bodies of all the Blattidae and in the termites. They also occur in the intestinal cells of ants. In fact, intra-cellular bacteroids are probably not at all unusual in multicellular organisms, and it seems likely that they are symbionts in all instances in which they are normal constituents of the cell. Symbiosis is of course very common in both the plant and animal kingdoms, but we are at present concerned only with intra-cellular symbionts which are not green and which have some useful function other than the obvious one that depends on the presence of chlorophyll.

Now, it is conceivable that there exist ultra-microscopic organisms which are akin to viruses but which are useful symbionts, and that these symbionts occur *universally* within the cells of larger organisms. We might call these supposed symbionts *viroids*. They would be analogous to the bacteroids above mentioned in that they would be of value to their host in connection with one or more cell processes. Ordinarily the biologist would be unaware of their existence. But if they should mutate, they might

conceivably give rise, for example, to the killer factor of Sonneborn (1945) in the cytoplasm of Paramecium, or to bodies that produce (or help to produce) the "adaptive enzymes" of Lindegren (1945) and Spiegelmann (1945), or to a virus.

We shall now consider the possibility that "viroids" are causally related to cancer. In view of the increasing number of cancers found to be caused by viruses, it is not at all impossible that *all forms of cancer are due to viruses.* On the viroid theory here being proposed, these viruses would ordinarily arise anew by mutation in each case of cancer from a viroid normally present as symbiont in all members of the host species. Carcinogenic agents would merely cause a mutation from symbiont viroid to pathogenic virus. Such carcinogenic agents might consist of special kinds of genes within the nucleus, in which case the cancer would be of the hereditary type; or, the carcinogenic agent might be some chemical or other environmental agent. Since viruses are known to have a high mutation rate under special conditions, viroids would also be expected to have.

The viroid theory would explain the fact that cancers might be due to a virus even though there was no infection from an outside source. It would also account for the widespread occurrence of cancer, since all people would normally carry the viroid and all might, therefore, under the proper conditions (environmental or genetic) come to carry the virus. The viroid theory would be in harmony with the fact that the virus might not necessarily be transmissible by inoculation, since the conditions might not be right in the new host for the growth of the virus. Or, it might not be readily possible to inoculate a cell from without, since the virus would normally never exist outside the cell and might rapidly be destroyed under the conditions of inoculation. Different cancer viruses undoubtedly differ in their capacity for artificial transferability.

A striking fact in connection with cancer is that a

chemical carcinogen can cause the production of the Rous sarcoma of fowl (as shown by McIntosh, 1933). Since the Rous sarcoma is known to be due to a virus, the carcinogen must be causing the virus to appear in normal fowl tissue. On the viroid theory this would be explained on the ground that the virus arose as a mutation from a viroid normally present in fowl, the mutation having been produced by the carcinogen. Another striking fact in line with the one just mentioned is that normal fowl tissue can cause the production of antibody against the Rous sarcoma. On the viroid theory this fact would be explained on the ground that viroid (in normal tissue) and virus (in the sarcoma) are very much alike, and that an antibody against the one would also be an antibody against the other. However, the antibody against the viroid would not be expected to be exactly like the antibody against the virus, since the two antigens (viroid and virus) would differ somewhat, and so the antibody formed against the normal tissue (supposedly containing the viroid) would not be quite as efficient against the virus as would be the antibody against the virus itself. This, in fact, is actually the case.

It is very unlikely that many cancers are due to nuclear gene mutation in the host. For, as shown by Auerbach (1940), working under Muller's direction, chemical carcinogens do not cause an increase in the mutation rate of genes in the nucleus of Drosophila cells. Yet carcinogens would be expected to cause such an increase if they often produced cancers by causing nuclear gene mutations in the host. Moreover, though it is now known that certain chemicals can produce mutations, yet chemicals in general are not very effective in artificially producing mutations, since as a rule they can not reach the nucleus and cause mutations without first doing serious damage to the cytoplasm, thus causing the death of the cell. On the other hand, the number of chemical agents that produce cancer is fairly large. On the viroid theory this might be explained on the ground that the viroids, being located in

the cytoplasm, would be more directly exposed to chemical agents than would the nucleus (protected as it is by the cytoplasm).

The viroids themselves may well be representatives of a group of organisms which at an early stage in evolution were free-living—the naked gene stage. Perhaps they were the dominant form of life in the Archeozoic. We can refer to these free-living organisms, together with the viroids and viruses (and bacteriophages) as the *Archetista* (sing., *Archetiston*). This group of organisms would be neither plants nor animals. Hence the Archetista would constitute a separate kingdom.

Possibly the higher plants and animals acquired many of their viroids when they were in the one-cell stage of evolution, since at this stage the Archetista might still have been existent as free-living organisms, and they might very well have become associated as symbionts with the one-celled organisms, both types of organisms having existed side by side in the same medium. The small size of the Archetista would have rendered them more adaptable than the bacteria as intra-cellular symbionts, especially in the earlier stages of evolution, when most cells were not as yet much larger than the bacteria themselves. Thus, in large measure, the Archetista would have pre-empted the field as intra-cellular symbionts. Hence their expected universality as symbionts in the larger organisms.

It is conceivable that the Archetista above assumed might in some cases have invaded the one-celled organisms as parasites and that they were then viruses, without at first being symbiotic viroids. They might next have become viroids through adaptation on the part of the host or of the virus or both. These viroids might then have become viruses again. Thus the evolutionary sequence would have been free-living Archetiston, virus, viroid and virus. It is, however, improbable that viroids would often have arisen from seriously pathogenic viruses (though they might have occasionally from mildly

pathogenic), since the affected hosts would have been as a rule exterminated before the virus could have changed to the viroid. More likely viroid would have arisen from free-living Archetiston without first going through a parasitic stage. The same sort of thing would apply to symbionts in general.

If the Archetista ceased to exist as free-living organisms at about the one-cell stage in evolution, and further, if the viroid theory is correct, then it seems probable that most of the present-day viruses were derived from viroids rather than from free-living Archetista, since it seems unlikely that the whole line of evolution from Ameba to man was parasitized by an unbroken line of viruses. More likely, the Archetista would first have become viroids, and then from time to time in the course of evolution some of them would have become viruses (as side branches, the main line of viroids persisting). But the Archetista might very well have continued uninterruptedly for long periods as viroids (from Ameba to man), since they would have had an indispensable role in the economy of the cell. Thus the viroids would have served as the "reservoir" for the viruses in the course of evolution. Some of the present-day viruses would have branched off from the viroid stock earlier in evolution than others, and accordingly the first would as a rule be much more different from the original viroid than would the second. Undoubtedly, the ordinary virus diseases, such as yellow fever and smallpox, are of comparatively ancient standing, and the viruses for these diseases are correspondingly ancient. We might refer to them as *paleo-viruses*. The cancer viruses, on the other hand, would, on the viroid theory, arise anew in each cancer case from a viroid, perhaps as the result of just a single mutation. They would be *neo-viruses*. Thus there would be a much closer kinship between a cancer virus and a viroid than between an ordinary virus (as for smallpox) and a viroid.

It has been assumed by Andrewes (1939), by Auerbach

(1940) and others that viruses might become latent or harmless, but that under stimulating circumstances they might become harmful, causing cancer and other diseases. Andrewes assumes further that latent viruses are of widespread occurrence. In this regard, they would resemble viroids, but in other respects they are not to be confused with the viroids here being assumed, since latent viruses would primarily be pathogenic organisms, not symbionts normally present in all members of the species and indispensable to the existence of the species. It is, of course, possible that a host might become immune to the effect of a virus and that it might still harbor the virus, which would then be latent; or that the virus might mutate to a harmless type and thus become latent. But such latent viruses would be rather exceptional; they would hardly be expected to be of universal occurrence, since if the virus infection was in the first place an accident, then most members of the species would not as a rule harbor the virus, and still fewer would harbor the latent virus. Moreover, if latent viruses were ordinarily the forerunners of cancer (as assumed by Andrewes), then they might also be expected often to be the forerunners of ordinary virus diseases. But carcinogens do not cause a flare-up of latent viruses that are not associated with cancerous growths, though they might be expected to if latent viruses were commonly the forerunners of cancer viruses. It might seem perhaps that a similar objection would apply to the viroid theory: why do not carcinogens sometimes cause non-cancerous virus diseases? As above mentioned, the non-cancerous virus diseases are of comparatively ancient standing—they are paleo-viruses—and on the viroid theory they would have undergone considerable evolution since their origin from viroids. A carcinogenic agent could therefore not very well produce them from a viroid at a single stroke. But, on the viroid theory, a cancer virus is a neo-virus—it is newly arisen from a viroid in each and every host that has cancer (unless the cancer has been artificially transferred). It

might therefore be due to a single mutation of a kind producible by a carcinogen. It is conceivable that incipient non-cancerous virus diseases are occasionally produced by carcinogens, but these would not be the ordinarily recognized virus diseases, such as yellow fever and smallpox, which originated a long time ago and undoubtedly have had a long evolutionary history.

The difference above referred to between latent virus and viroid would have a practical application in the treatment of cancer. For, any treatment which killed the cancer virus might be expected to kill its predecessor (latent virus or viroid, as the case might be), since the virus and its predecessor would not be so very different. Now if the predecessor were a latent virus, the treatment would not necessarily harm the host; but in case it were a viroid, then damage might be done to the viroid predecessor still present in the unaffected tissues of the host, and so it might be injurious, even to the extent of being fatal, to the host. On the viroid theory, the treatment of cancer would in general be subject to the limitation that the treatment must not damage the viroid predecessor.

It is possible that a viroid (A) in one species might be artificially transferable to some other species with a somewhat different type of viroid (B) and that A might then spread in the inoculated individual, in which it might now act not as a symbiont but as a parasite either because it displaced B and could not itself continue the specifically useful function of B or because it was directly injurious to its new host. In either event it would act as a virus in its new host. Johnson (1945) has found that most American potatoes normally contain an agent which is infectious in tobacco. This, on the present theory, might be an organism which was a viroid in potatoes but a virus in the tobacco.

Plastids (or rather the plastogenes which produce them) are peculiar to plants and are probably not modified viroids. They are located in the cytoplasm as the result of the fact that this is the most advantageous loca-

tion for their reproduction and efficient operation. It is probable that they were never in the nucleus and that, as suggested by Muller (1929), they antedate the nucleus and simply have not become incorporated in it. They differ from other genes in not being concerned directly with development but with the physiological process of building up carbohydrates. It is hardly necessary to mention here the old idea that plastids came into plants as originally independent invading green organisms, like the symbiont algae in lichens. There is no evidence for this view; nevertheless it is conceivable.

Plastids sometimes lose their color because of a mutation in the plastogene, but they might also lose it because of a virus infection, or more often, because of a mutant nuclear gene. In the latter case, the nuclear gene might cause the plastogene to mutate to the white type, or it might interfere with the functioning of the plastogene without necessarily causing it to mutate.

The idea that viruses arise from normal constituents of the cell is not new. In particular, Darlington (1944) has proposed that ordinary cell proteins might become self-reproducing bodies by combining with nucleic acid, and that they might then become plasmagenes, or that they might become viruses and cause cancer as well as infectious diseases. But it has never been shown that ordinary cell proteins can become self-reproducing. Darlington's view, in fact, seems akin to the ancient theory of spontaneous generation, since our only criterion for distinguishing between lifeless and living substance of the most primitive kind is a difference in their capacity for growth and mutation, both of which capacities are supposed by Darlington to be conferred upon lifeless proteins by nucleic acid. The viroid theory, on the other hand, calls for nothing new in principle, since symbiosis is known to be of widespread occurrence in both the plant and animal kingdoms.

The viroid theory explains the fact that certain bodies have the properties not only of normal cell constituents

but also of independent organisms. For, viroids would have a certain capacity for evolution independently of their host, since they are separate organisms. They could, therefore, presumably give rise (through mutation and natural selection) to viruses which could live for a while in an intermediate host very different from the principal host. Thus, they might become transmissible by an insect vector. This would hardly be expected of a gene which belonged to the host cell, since genes (and the enzymes they produce) are parts of a complicated close-knit organization. This difference in capacity for transmission constitutes a real distinction between a viroid and a plasmagene, if by the latter we mean a body that is part of the gene system of the host cell. A viroid is therefore not simply a plasmagene with another name; and the possibility that viroids exist will have to be taken into account in any discussion of inheritance involving cytoplasmic bodies that are ultra-microscopic in size.

LITERATURE CITED

Andrewes, C. H.
1939. *Proc. Roy. Soc. Med.*, 33: 75–86.
Auerbach, C.
1940. *Proc. Roy. Soc. Edinb.*, 60: 164.
Brues, C. T., and Ruth C. Dunn
1945. *Science*, 101: 336–337.
Darlington, C. D.
1944. *Nature*, 154: 164–169.
Johnson, J.
1945. Research Bull. No. 3 of Agri. Exp. Sta. of U. of Wis.
McIntosh, J.
1933. *Brit. Jour. Expm. Path.*, 14: 422.
Lindegren, C. C.
1945. *Ann. Missouri Bot. Garden*, 32: 107–123.
Muller, H. J.
1929. *Proc. 4th Int. Congr. Plant Sci.* (*Ithaca*), 1: 897–921.
Sonneborn, T. M.
1945. *Ann. Missouri Bot. Garden*, 32: 213–221.
Spiegelmann, S., C. C. Lindegren and G. Lindegren
1945. *Proc. Nat. Acad. Sci.*, 31: 95–102.

A SYSTEM OF SCORING LINKAGE DATA, WITH SPECIAL REFERENCE TO THE PIED FACTORS IN MICE

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(1) THE LINKAGE OF THE TWO PIED FACTORS s AND W

EARLY last year Gates and Pullig (1945) reported a significant linkage between dominant spotting, *W*, and hairless, *hr*, in *Mus musculus*. Of 605 mice classified in a backcross in coupling, 255 were recombinants, while 350 were of the parental combinations. The finding is statistically significant, and the recombination fraction indicated is 42.15 per cent.

Many years earlier Snell (1931) had discovered that *hairless* was closely linked with the recessive spotting factor, *s*. His data consist of 193 young classified from doubly heterzygous females backcrossed, of which 19 were recombinants, and 155 from doubly heterozygous males, crossed with recessive pied mice heterozygous for hairless. This cross was therefore a backcross for *s* but an intercross for *hr*. There were recorded:

	Self-normal	Self-hairless	Pied-normal	Pied-hairless
	77	1	38	39
Expected	$2-y$	y	$1+y$	$1-y+4$

Difficulty appears to have been encountered in classifying the pied factor, *s*, but accepting the data as they stand the recombination fraction indicated is about 8.4 per cent.

In spite of frequent statements to the contrary, therefore, it appears that the two spotting factors *W* and *s* must be linked. I have, therefore, made a compilation of records in my own stock, and find of 1,181 mice bred from backcrossing double heterozygotes, the grandparental combination appears in 636, while 545 are recombinants. Nearly all (1,168) of these offspring were from the coupling backcross $WS/ws \times ws/ws$; for this reason, these records, while showing a significant deviation in

the direction to be expected with linkage, would scarcely, if they stood alone, be decisive, since with backcrosses made in one way only the results may be disturbed by small differences in viability.

It is moreover, of some interest to ascertain how, with linkage demonstrable without very extensive tests, independent segregation should have been confidently asserted for these factors. Was my experience exceptional, in which case a compilation of the data, old and new, should show significant heterogeneity, or did the data published by others contain indications in harmony with mine, and if so by what fault in compilation had its indications come to be overlooked?

(2) Scoring Linkage Data

The principles of statistical estimation have often been illustrated by means of linkage data (Fisher, 1928–45) and Mather (1938) in his useful book "The Measurement of Linkage," gives a valuable account of how to proceed. In studying the problems which arise in the detection of linkage in man (Fisher, 1935), I was led to put forward a method of scoring, which overcomes the difficulties of this intricate material, and seems fitted for more general use in genetics, where compilations and questions of homogeneity and of pooled estimates come into view. It serves well also to illustrate in concrete terms the ideas of the theory of estimation, which, developed as an abstract mathematical discipline, have not become fully familiar to practical experimenters.

If $c_1, c_2, \ldots, c_s$ are a series of scores assignable to different bodies of data, with a view to testing their deviations from any proposed hypothesis, and if $b_1, b_2, \ldots, b_s$ are the sampling variances of these scores, then it is well known that, the several bodies of data being independent, it follows that the total score,

$$C = S(c),$$

has a sampling variance defined by,

$$B = S(b).$$

So far as this condition is concerned we might multiply any score by an arbitrary factor k, and the corresponding variance by k^2, without altering the position. Clearly $S\left(\frac{c^2}{b}\right)$ will be distributed as χ^2 for s degrees of freedom.

If estimates $x_1, \ldots, x_s$ derived from independent bodies of evidence are to be considered, it is known that the joint estimate of least sampling variance is

$$S(wx)/S(w)$$

where the weights are inversely proportional to the sampling variances of the estimates. Consequently, if the scores c can be so chosen that

$$x = c/b$$

is a consistent estimate of some quantity to which an incorrect value may have been assigned in the hypothesis under examination, then the sampling variance of x is

$$\frac{1}{b^2}V(c) = \frac{1}{b},$$

the weights will therefore be proportional to b, and the efficient joint estimate will be

$$S(bx) + S(b) = C/B.$$

Since $\frac{C}{B}$ is efficient we may now take $S\left(\frac{c^2}{b}\right) - \frac{C^2}{B}$ as a measure of heterogeneity, it being distributed by errors of random sampling in a χ^2 distribution of $s-1$ degrees of freedom.

We shall now show how from any body of data quantities of c and b can be calculated fulfilling these two conditions, and therefore providing the proper system of scoring. For example, let us consider Snell's data given above for 155 mice obtained in a partial backcross for *hr* and *s*.

The expectations of the four observable classes are expressed in terms of the number of mice in all classified, and the undetermined recombination fraction, y. Then for these four classes we have the expectations

$$m = \frac{155}{4}(2-y,\ y,\ 1+y,\ 1-y)$$

From these we may obtain the differential coefficients with respect to y, adding necessarily to zero,

$$\frac{\partial m}{\partial y} = \frac{155}{4}(-1,\ 1,\ 1,\ -1).$$

In this case these are independent of y, but more generally they will be functions of y, the parameter chosen for testing. The scores corresponding with the four phenotypes distinguishable are taken to be the values of $-\frac{\partial m}{\partial y} \div m$. Thus for testing independence, $y = \frac{1}{2}$, the four scores are

$$\frac{2}{3}, -2,\ \frac{-2}{3}, 2,$$

or in general

$$\frac{1}{2-y}, -\frac{1}{y}, -\frac{1}{1+y\chi}, \frac{1}{1-y\chi}.$$

Hence, if the numbers of individuals observed in these four classes are

$$a_1, a_2, a_3, a_4,$$

the total score for the experiment is

$$c = \frac{a_1}{2-y} - \frac{a_2}{y} - \frac{a_3}{1+y} + \frac{a_4}{1-y};$$

and since the expectations of the observed frequencies a are the values m, it follows that the expected value of the score is always zero.

Since the score is a linear function of the frequencies its sampling variance is easily calculated (Fisher, 1928–45, section 55) to be

$$i = S\left\{\frac{1}{m}\left(\frac{\partial m}{\partial y}\right)^2\right\}$$

a quantity known in the general theory of estimation as the amount of information about y supplied by the sample.

Further, if instead of m we substitute $m + \frac{\partial m}{\partial y} Sy$ for a in evaluating the mean value of the score, we obtain instead of zero

$$S\left\{\frac{1}{m}\left(\frac{\partial m}{\partial r}\right)^2\right\}\partial y = i\partial y,$$

showing that the correction to the value of y provisionally adopted, indicated by the sample observed, will be consistently estimated by the ratio

$$\frac{1}{m}\frac{\partial m}{\partial y} + i = b/c.$$

Hence the system of scores proposed with the corresponding amounts of information will satisfy all the requirements laid down. It should be noticed that the quantities i fulfill three functions, (1) as the amounts of information they supply a measure of the value obtained for the time and labor expended upon each batch of data, (2) they are divisors of the scores appropriate for obtaining a more correct estimate than that provisionally adopted, (3) they are the sampling variances of the scores obtained, by means of which the significance of any deviation or heterogeneity may be tested.

For the 155 mice chosen as example, the amount of information at any chosen value of χ is clearly

$$\frac{155}{4}\left(\frac{1}{2-y}+\frac{1}{y}+\frac{1}{1+y}+\frac{1}{1-y}\right)$$

$$\frac{155}{2}\ \frac{1+2y-2y^2}{(2-y)y(1-y^2)}$$

With $y = 9$ per cent. this is 528.9764; similarly, the 193 mice from the double backcross give

$$\frac{193}{y(1-y)} = 2356.5324,$$

or, in all, 2885.5088 units of information reckoned at 9 per cent.

At the same percentage the total score of the two sets of data is + 17.29546, so that judging from these data alone the efficient estimate must be about 0.6 per cent.

lower than the recombination fraction adopted, *i.e.*, about 8.4 per cent. Since, however, $(17.3)^2$ is quite small compared with 2,885, the efficient estimate will differ from 9 per cent by much less than its standard error of estimation, so that, pending further data, the calculation at 9 per cent. will tell us all we could learn from a recalculation nearer to the efficient estimate.

For testing the significance of linkage, data will be scored at $y = \frac{1}{2}$; but for examinations of homogeneity and

TABLE 1

SCORES FOR SIGNIFICANCE OF LINKAGE, IN VARIOUS TYPES OF MATING INVOLVING *W* AND *s*

A

Signs appropriate for coupling, reverse for repulsion

	Double backcross	*W* backcross *s* intercross	*W* intercross *s* backcross	Double intercross
Self *wS*	−2	−2/3	−2	−4/3
White *Ws*	−2	−2	0	0
Pied *Ws*	+2	+2/3	0	0
Pied *ws*	+2	+2	+2	+4
Mixed pied	+2	+1	+2/3	+4/7
Information per mouse, i	4	4/3	4/3	16/9
Information pieds mixed, i	4	7/6	8/9	40/63

B

For any given recombination fraction y. For repulsion reverse the signs and substitute $1-y$ for y.

Self wS	$-2/(1-y)$	$-1/(1+y)$	$-1/y$	$-2(1-y)/y(2-y)$
White Ws	$-2/(1-y)$	$-1/y$	0	$-(1-2y)/(1-y)$
Pied WS	$2/y$	$1/(2-y)$	0	$(1-2y)/(1-y+y^2)$
Pied ws	$2/y$	$1/(1-y)$	$1/(1-y)$	$2/(1-y)$
Mixed pied	$2/y$	$2/(3-2y)$	$1/(2-y)$	$(4-6y)/(3-Dy+3y^2)$
Information	1	$(1+2y-2y^2)$	1	$2(4-13y+16y^2-6y^3)$
per mouse	$y(1-y)$	$2y(1-y^2)(2-y)$	$3y(1-1)$	$y(1-y)(2-y)(1-y+y^2)$
Information	1	$(3+8y)$	2	$2(12-45y+54y^2+19y^3)$
pieds mixed	$y(1-y)$	$4y(1+y)(3-2y)$	$3y(2-y)$	$3y(1-y)(2-y)(3-4y+3y^2)$

the combination of evidence from different sources, it should be rescored, using a value sufficiently close to the efficient estimate. Scores and amounts of information per individual classified, appropriate to $y = \frac{1}{2}$, are given for a number of common cases in Table 1.

With backcrosses fully classified the score is twice the difference between the grand-parental and the recombinant classes, while the amount of information is four times the number of individuals classified. Thus with the evidence for *W* and *hr* we have at 50 per cent.

	Score	Information
	190	2,420

where

$$\chi^2 = (190)^2/2420 = 14.9174,$$

showing the discrepancy to be clearly significant. Rescored at 44 per cent. we find

	Score	Information
$-255/\chi y$	−579.54545	
$+350/(1-y)$	625.	
	+ 45.45455	$2455.357 = 605/y(1-y)$

χ^2 is now less than unity, so there is no significant discrepancy, although a recombination fraction nearly 2 per cent. less than that adopted is indicated by the data.

TABLE 2

	Score 50 per cent	I_{50}	Score 46 per cent	I_{46}	Total mice
Little (1915)					
Repulsion intercross ..	+ 2.2857	36.190	+ .2219	27.777	57
Repulsion backcross ..	+ 40.0000	512.000	+ 19.6458	515.298	128
Little (1917). Non-yellows only.					
Repulsion backcross ..	+ 6.0000	548.000	− 16.3445	551.530	139
Dunn (1920)					
Repulsion intercross ..	+ 8.5714	38.730	+ 3.8852	29.726	61
Repulsion backcross ..	+ 26.0000	1796.000	− 46.1350	1807.568	449
Keeler (1931)					
Coupling intercross ..	− 1.3333	48.000	− 3.0728	51.436	27
Fisher (new data)					
Backcross	+ 182.0000	4724.000	− 7.0044	4754.429	1181
	+ 263.5238	7702.920	− 48.8038	7737.764	
χ^2, deviation	9.0154		.3078		
Correction	− 3.421 per cent		+ .631 per cent		
Estimate	46.579 per cent		46.631 per cent		

(3) Data on *W* and *s*

Much of the early data on *W* and *s* is complicated by the fact that difficulty was encountered in distinguishing *WwSs* dominant pied from *wwss* recessive pied. The lethality of *W* also causes intercrosses involving this factor to give unusual ratios. The method of scoring set out in Section 2 is however applicable to all cases, if the true expectations of each distinguishable phenotype are used.

Table 2 gives the scores and corresponding amounts of information for seven lots of data on *W* and *s*. scored at 50 per cent. and at 46 per cent. Except for the very

small batch of Keeler (1931) each lot favors linkage, as shown by the positive score at 50 per cent. This should have prevented the assertion that independence had been demonstrated, although in the absence of the new material now added, the evidence was somewhat insufficient to justify the existence of linkage being positively asserted. It is fortunate that the bulk of the data available in addition to my own consists of tests in repulsion, for these are what is required to supplement the coupling data I have to add.

The total score at 50 per cent. is clearly significant with χ^2 exceeding 9 units. The recombination fraction indicated by the data collected is 46.631 per cent., somewhat higher than might be expected from the estimated value of 42.1 per cent. for *hr* and *W*. It is clear, however, that *hr* lies between the loci of the two pied factors.

(4) The Combination of the Data Subject to Restrictions

When data exist for the linkage relations of three or more loci, it is desirable to place them on a linkage map, taking into account all the evidence at any one time available. Such mapping is, of course, provisional, and will be expected to be made more precise as more data is brought into account. The relation between recombination fraction and map distance is, however, somewhat obscure, and it is of some importance to be in a position to form an opinion as to whether any particular formula is or is not in harmony with the facts.

The most promising general approximate relationship so far proposed is that of Kosambi (1944) namely, if x is the map distance and y the recombination fraction

$$tanh\ (2x) = 2y$$

or

$$4x = \log\,(1+2y) - \log\,(1-2y)$$

using natural logarithms.

If y_1 and y_2 are the recombination fractions corresponding to two adjacent segments, it follows from Kosambi's formula that

$$y_{12} = \frac{y_1 + y_2}{1 + 4y_1y_2}$$

is the recombination fraction for the two extreme loci.

If, therefore, we score y_1 at 9 per cent. and y_2 at 44 per cent. we must for consistency with this formula score y_{12} at 45.75276 per cent, moreover,

$$\frac{\partial y_{12}}{\partial y_1} = \frac{1 - 4y_2^2}{(1 + 4y_1y_2)^2} = .1681210,$$

and

$$\frac{\partial y_{12}}{\partial y_2} = \frac{1 - 4y_1^2}{(1 + 4y_1y_2)^2} = .7210722.$$

Since then a unit change in y_1 produces a change of only about .168 in y_{12} we may use these fractions to combine the evidence supplied by direct experimentation with y_{12}, with that available for y_1, in comparing the entire evidence available with the proposed value for y_1.

Now at 45.75276 per cent. the score for y_{12} will be approximately

$$-48.8038 - (.0024724)(7737.764) = -67.9346$$

and this is to be multiplied by .168121 in the combined evidence; the amount of information will be multiplied by the square of the same factor, giving the complete comparison:

COMPARISON FOR INTERVAL $hr - s$

	SCORE	INFORMATION
From *Ws*	−11.4212	218.705
From *hr s*	+17.2955	2885.509
	+ 5.8743	3104.214

A similar comparison for the interval $hr - W$ gives

	SCORE	INFORMATION
From *Ws*	−48.9858	4023.213
From *Whr*	45.4545	2455.357
	− 3.5313	6478.570

Both comparisons have therefore been brought to a fair agreement by the values adopted. In order to use the discrepancies still remaining to adjust the recombination fractions to a more perfect agreement, we take into account the fact that the two discrepancies are not now independent, but that the full expression for the information has the product term

$$(7737.764)(.168121)(.7210722)$$

which is the geometric mean of the two values already used, or numerically

$$938.029.$$

With this value we can write the equations

$$\begin{aligned} 3104.214\partial y_1 + 938.029\partial y_2 &= -5.8743 \\ 938.029\partial y_1 + 6478.570\partial y_2 &= +3.5313 \end{aligned}$$

where ∂y_1 and ∂y_2 stand for the adjustments required in the recombination fractions adopted. The solution of these equations is

$$\begin{aligned} \partial y_1 &= -.002151, \\ \partial y_2 &= +.000875. \end{aligned}$$

A good fit may therefore be expected from the values

s hr	8.78 per cent	$\frac{\partial y_{12}}{\partial y_1}$	.1667801
hr W	44.09 per cent		
s W	45.78107 per cent	$\frac{\partial y_{12}}{\partial y_2}$	.7266928

Then for *Ws* we have

Score	Information	χ^2
−65.7441	7737.764	.5586,

for the interval *s hr*

	Score	Information	χ^2
From *s W*	−10.9648		
From *s hr*	11.0459	2909.9	.0419,
	.0811		

and for the interval *hr W*

	Score	Information	χ^2
From *s W*	−47.7758		
From *hr W*	47.6436	2454.3	.9249
	− .1322		1.5254

The discrepancy of the observations from Kosambi's formula is therefore measured by $\chi^2 = 1.5254$ for one degree of freedom. This point can, of course, only be tested after bringing about a fairly close adjustment.

The map lengths calculated from the recombination fractions obtained are

	Recombination Per cent	Map distance Centimorgans
s hr	8.78	8.87
hr W	44.09	69.18
s W	45.78	78.05

SUMMARY

(1) Gates' and Pullig's discovery of linkage between hairless (*hr*) and dominant spotting (*W*) shows that, contrary to previous statements, *W* and *s* also must be linked.

(2) New data are given showing significant linkage, and a review of the data previously available shows it to be homogeneous and uniformly to favor linkage; previous compilations have therefore been misleading.

(3) A method is given of scoring linkage data so that the evidence available from data of various kinds can easily be combined, and its heterogeneity examined.

(4) The method is illustrated with the problem of fitting consistent map distances using Kosambi's formula.

LITERATURE CITED

Dunn, L. C.
1920. AM. NAT., 54: 465–495.
Fisher, R. A.
1928–45. "Statistical Methods for Research Workers." Edinburgh: Oliver and Boyd.
1935. *Annals of Eugenics*, 6: 187–201.
Gates, W. H. and T. Pullig
1945. Abstract. *Genetics*, 30: 4.
Keeler, C. E.
1931. *Proc. Nat. Acad. Sci.*, 17: 101–102.
Kosambi, D. D.
1944. *Annals of Eugenics*, 12: 172–175.
Little, C. C.
1915. AM. NAT., 49: 727–740.
1917. *Genetics*, 2: 433–444.
Mather, K.
1938. "The Measurement of Linkage in Heredity." London: Methuen.
Snell, G. D.
1931. *Genetics*, 16: 42–74.

OBITUARY

BERTRAM GARNER SMITH, 1876–1945

DR. E. W. GUDGER
AMERICAN MUSEUM OF NATURAL HISTORY

DR. SMITH was born October 7, 1876, at Painesville, Ohio, the son of Albert W. and Ella Garner Smith. He died of a heart attack at his home in Albuquerque, N. M., July 30, 1945. He is survived by his wife and a daughter. He was of New England ancestry, through his grandmother Smith, from the Mortons who settled New Salem, Mass., about 1660.

When Smith was about two years old, his parents moved to Youngsville, Warren Co., northwestern Pennsylvania. There he received his early education, graduating from high school in 1893. In 1894, he entered the Pennsylvania State Normal School at Edinboro and graduated in 1896. For the next two years he taught in the public grade schools of his section of Pennsylvania. From 1899 to 1902, during the winters, he taught the sciences in the High Schools of Warren, Dubois, and Corry, Pa., and between times attended the summer sessions of Cornell University. In 1903, he matriculated at the University of Michigan, where he was assistant in zoology to Professor J. E. Reighard 1904–07, and from which he graduated A.B. in 1907.

He was instructor in biology at Lake Forest College in the spring of 1907, in zoology at Syracuse University 1907–09, and at Wisconsin in 1909–11. In 1911, he entered Columbia University as a graduate student in zoology under Dr. Bashford Dean, and because of much published research, he was able to take his Ph.D. in 1912. From this year's work stemmed a lifelong friendship with Dr. Dean. From 1912–16, he was assistant professor of zoology at Michigan State Normal College and associate professor 1916–21. From 1921 to 1930 he was associate professor of anatomy in New York University

BERTRAM GARNER SMITH

Medical College and professor of anatomy from 1930 until his retirement in September 1942.

Over the years 1906–1929, Smith's scientific work was chiefly done on amphibians. Of his 49 published papers, 22 were on members of this group, and 13 of these dealt with the giant salamander, *Cryptobranchus allegheniensis*. His interest in this dates from boyhood, when, fishing in the stream near his home, he would frequently catch a Cryptobranchus instead of a fish. Thus, when he learned of the importance of this animal from a zoological point of view, he knew where to find it. The breeding season and habits of this amphibian, sought for almost a generation, were a mystery until it was discovered that, unlike other amphibians, it breeds not in the spring but in the fall. Smith studied its habits and found how oviposition and fertilization are effected. His field observations ranged from 1905–1911, and his laboratory work from 1906–1929.

The difficulties of the field work of collecting and "fixing" the egg and life history stages were great. But quite as great were those of the laboratory work of embedding and sectioning these yolk-laden amphibian eggs averaging 6.2 mm. in diameter and exceeded in size only by those of *C. japonicus* (*c.* 7 mm. in diameter). Smith was a good artist and his papers are illustrated by his own drawings and photographs. The work on his articles, from start to finish, was done with his own hands. Unlike many researchers, he never had the help of assistants.

Smith's thirteen papers on the natural history and embryology of Cryptobranchus (published mainly in the *Biological Bulletin* and *Journal of Morphology*), range in date from 1906–1929. They comprise 484 pages and 590 drawings and photographs. Even a general examination of his papers on Cryptobranchus reveals what a prodigious amount of meticulous histological work he did on the development stages of these huge eggs and early embryos. I do not recall any vertebrate whose natural

history and embryology have been more thoroughly and successfully studied. These studies, together with those on Amblystoma and Necturus, comprise the major interest of the first period of his scientific activities.

The second period of Smith's productive scientific work began shortly after the death (December 6, 1928) of his teacher at Columbia, Dr. Bashford Dean. An organization of Dr. Dean's associates, students, friends and family was set up to establish memorials to him. Bronze plaques were cast and mounted in the American and Metropolitan Museums. Then came the question of what to do with four sets of splendid drawings (some in color) of certain archaic fishes—myxinoids and sharks—made for reproduction by lithography, and it was decided that these should be published as a Memorial Atlas under the direction of Dr. Smith and the writer (as editor).

After much thought, I determined that, instead of a Memorial Atlas, we would publish a Memorial Volume if I could have Smith's help, since his training in embryology and anatomy would be invaluable. And when next he came to my office, I announced my proposed plan and without a moment's hesitation he held out his hand and said—"I came to tell you just that thing. I, too, owe it to Dr. Dean." Nothing more clearly illustrates the spirit of the man. Then began work that covered 13 years and in which we did five of the eight articles in the volume. This was especially hard for Smith, who was carrying a full teaching load in the department of anatomy of New York University Medical College. Furthermore, it was time-consuming for him to come to and return from the American Museum. But for all that—he came.

In 1931 and 1933, we published two joint papers—one on the natural history of the frilled shark. Then came the long hard pull for more than three years in which Smith prepared his great "Anatomy of the Frilled Shark, *Chlamydoselachus anguineus*," (published 1937) of 190 quarto pages, 7 half-tone plates and 128 text-figures. In shark anatomy this book, on one shark only, measures up

to J. F. Daniel's "Elasmobranch Fishes" (3rd. ed., 1934, octavo, 322 pp., 270 figs.).

But even this was equalled by the final article in the Memorial Volume, "The Heterodontid Sharks: Their Natural History and the Development of *Heterodontus japonicus*, Based on Notes and Drawings by Bashford Dean."—138 quarto pp., 7 lithographed plates (5 in natural color) and 69 text-figs. This I (as editor) had held for Smith and for the final article in the Memorial Volume, and when (October 1, 1942) I handed him the first copy from the binder, I said, "This is the high note of the Volume, and also of your scientific writings." But little did I apprehend how true the latter statement was to be.

Dr. Smith retired from his work in N. Y. University, September 1, 1942, settled up his affairs in the East and presently went to Albuquerque, N. M., where he bought a house and settled down to adapt it and the grounds (with his own hands) to make it a home. Things went well until in the Spring of 1945 he began to have heart attacks, to one of which he succumbed on July 30. Thus passed a fine man, who made elaborate studies of the natural history and embryology of one of the least known American amphibians. Later he made similar additions to our knowledge of the natural history, anatomy and embryology of two archaic sharks. These notable monographs, the outcome of ability and persistent hard work, are the monuments in American Zoology to Dr. Bertram Garner Smith.

REVIEWS AND COMMENTS

EDITED BY PROFESSOR CARL L. HUBBS

In these reviews and notices of current biological publications emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution. REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as indicated, all items are prepared by Dr. Carl L. Hubbs, Scripps Institution of Oceanography, University of California, La Jolla, California. All opinions are those of the reviewer.

Mammals of Nevada. By E. RAYMOND HALL. Berkeley and Los Angeles: University of California Press, 1946: i–xi, 1–710, figs. 1–485 (plus 55 figs. and 38 maps, unnumbered), frontisp. + pls. 1–11, charts 1–2. $7.50.

HALL's long-awaited *magnus opus* on the mammals of Nevada is finally published. It is a large and beautifully printed monograph, crammed with factual material and a gratifying abundance of illustrations. In addition, both in the introduction and through the systematic text, there is presented the speciational philosophy of the author and a considerable amount of general interpretation and discussion. There is a brief consideration of the adaptations of the mammals to the climatic conditions of the state—varying from heavy snowfall to extreme aridity. Distributions are summarized according to floral belts, life-zones, faunal areas and centers of differentiation. The life-zone concept is by no means abandoned, but distributional factors other than temperature are also stressed. Edaphic, vegetational and humidity factors are given considerable emphasis as of controlling significance in some species. Faunal areas are characterized by the occurrence of full species, differentiation centers by distinct subspecies. As might be expected difficulty was experienced in ranking certain regions on this basis. Peculiar soil conditions that were laid bare and made available to mammalian incursion by the recession of Lake Lahontan are suggested as one basis for subspeciation. Clines are discussed and very briefly illustrated, with little quantitative data. In fact there is a rather glaring deficiency of statistical analysis.

In the introductory section on speciation, subspecies are contrasted with species largely on the basis of the greater sharpness of the characters of species at the boundaries of their range. Subspecies are wisely regarded as shorter-lived than species, typically as relatively ephemeral, for otherwise the world would be overrun with incipient species. Groups vary widely in plasticity. As usual in mammalogy, the criterion for ranking a form as a subspecies or as a species is areal intergradation. But intergradation is seldom accorded critical analysis in text or maps and it is indicated that, because of their limited and distinctive habitats, related kinds very often fail to come into actual contact. When the ranges are of the insular type decision as to ranking is made on a morphological basis, depending on whether or not the differences are as great as the maximum displayed by forms which can and do intergrade geographically. Many will argue this point. A recent mammalian species is defined as "an evolved (and often evolving), reproductively isolated natural population in which individuals of similar age and sex (in at least one group of one sex) possess in common certain heritable, morphological characters that are distinctive." Further, "in these higher vertebrates the courses of evolution, both divergent and monotypic [transformational] . . . are maintained, controlled and guided by the careers of changing and shifting environments." Evidence that is included, but not singled out, confirms the view that pelage color is largely determined by selection in accordance with soil colors.

A great quantity of natural history data is mixed in the systematic discussions. Included also is a general discussion of man as the most effective enemy of mammals. Under *Canis latrans* there is a 22-page treatment of coyote control in which there is presented a new, compromise plan for dealing with the coyote problem.

Greatest emphasis is usually given to the detailed distribution of each form. Maps show the general range of

the species (as an insert) and, for Nevada only, the inferred distributional limits and the record stations. The very extensive material is listed. Subspecies are compared in text and in many tables of measurements. The author, his associates and the press are all to be congratulated on the thorough, high-quality presentation of an immense amount of worthwhile information and interpretation.

Marine Microbiology: A Monograph on Hydrobacteriology. By CLAUDE E. ZOBELL. Foreword by Selman A. Waksman. Waltham, Mass.: Chronica Botanica Co. (New York City: G. E. Stechert and Co.) 1946: i–xv, 1–240, figs. 1–12. $5.00.

THIS exposition of the nature of marine microorganisms and of their function in the biology of the sea is also a valuable key to the widely scattered literature of the field. Many investigations concerning bacteria, yeasts and molds found in marine environments have been reported, but little attempt at collecting and condensing these reports has heretofore been made.

Introductory chapters survey the history of marine microbiology and give an account of the factors which characterize the marine environment. Sample collection and the enumeration of marine bacteria are discussed in detail. The emphasis upon methodology is deserved because of the demands in technique peculiar to the marine bacteriologist.

The many environmental factors which affect the distribution of bacteria in the sea are discussed at length and in the light of their interrelationships. Thus are described distinct effects of variations in phytoplankton and zooplankton numbers upon bacterial populations. The quantitative distribution and physiological diversity of microorganisms inhabiting sedimentary materials are discussed, and the effects of such organisms upon their environment are accentuated.

The nature and occurrence of aquatic yeasts and molds receive comparatively brief attention. However, this section is well documented and the reader is referred to valu-

able sources of information, such as Sparrow's extensive work on aquatic Phycomycetes.

Several chapters are devoted to the physiological activities of marine bacteria. These deal with the transformation of organic matter and with the cyclical regeneration of nitrogen, sulfur and phosphorus. The stress upon such activities is gratifying, for these constitute the most important function of bacteria in the sea. In this discussion the author points to several means by which bacterial activity is of great importance to the larger organisms. Included are the decomposition of organic matter into the materials of the photosynthetic process, the accumulation of dilute organic compounds into more concentrated bacterial cell substance assimilable by various animals, the symbiotic aid of bacteria in the digestive activities of marine animals and the primary production of organic matter by autotrophic bacteria. The description of bacterial metabolism and of its far-reaching influences is followed by a summarization of both the beneficial and harmful effects of marine bacteria upon plant and animal life.

Sanitary aspects of marine microbiology are outlined and evidence pertaining to the adventitious character of human pathogens in sea water is given. The economic value of marine microorganisms is indicated and related largely to such deleterious influences as fouling of submerged surfaces, destruction of nets and spoilage of marine food products. The monograph is concluded with an abridged description of the microbiological features of inland waters, both fresh and saline.

The text is followed by a bibliography of more than 600 titled entries. There are also author and subject indices. The book is well written. It provides a helpful and stimulating tool to research and fills a serious gap in the literature of microbiology.—William D. Rosenfeld, *Scripps Institution of Oceanography, University of California, La Jolla, California.*

SHORTER ARTICLES AND DISCUSSION

THE RELATIONSHIP OF X-RAY INDUCED RECESSIVE LETHALS TO CHROMOSOMAL BREAKAGE

THE work of Bauer, Demerec and Kaufmann (1938) and of Bauer (1939) indicated an exponential relationship of observed chromosomal rearrangements to x-ray dosage. Oliver (1932) and Timoféeff-Ressovsky (1939) demonstrated a linear relationship between dosage and the number of recessive lethals produced.

Of special interest is the relationship between recessive lethals and chromosomal rearrangements. In those experiments where Oliver (1932) detected lethals on the X chromosome and then subsequently tested them for association with chromosomal rearrangement he found the following. Not only did the per cent. of lethal cultures showing rearrangement increase with dosage, but in almost all cases it was impossible to separate the lethal from the rearrangement by crossing over. The same type of observation was made by Demerec (1937). After examining a number of lethal cultures for their association with chromosomal rearrangements, Demerec found an increase with dosage in the per cent. of lethals which were associated with rearrangements. He also found that when both lethals and rearrangements occurred on the same X chromosome, more than 90 per cent. of the cases showed the lethal to be at a breakage point of the rearrangement.

Additional data were desired to test this association between lethals and rearrangements as an aid in postulating a mechanism of x-ray action in the production of recessive lethals. To this end, irradiations of *Drosophila melanogaster* were performed at two doses previously uninvestigated, 1,000r and 5,000r. Males from the Canton S wild-type stock were x-rayed and mated to virgin *CIB* females from Muller's *CIB* stock. Employing the *CIB* technique, lethals were detected in the F2 generation. Lethal cultures were then tested in a crossover generation for their association with chromosomal rearrangements. A chromosomal rearrangement was detected as a reduction in crossing over of 50 per cent. or more in either or both regions of the X chromosome tested.

Results

The results of these experiments are summarized in Table 1. It is clear that the lethal rate increases linearly with dosage. There were twelve cases in which lethals and rearrangements were associated, two came from 1,000r treatments and the remainder from treatments at 5,000r. Of a total of forty-three recessive lethals which could be tested none showed a chromosomal rearrangement unassociated with a lethal. These cases included one from the controls, sixteen from the 1,000r treatments, and twenty-six from the 5,000r treatments. To calculate the valid per cent. of lethals associated with rearrangements it was necessary to

TABLE 1

Summary of Recessive Lethal Data and of Lethals Associated with Chromosomal Rearrangement

Series	Number of fertile F_2 cultures	Number of confirmed lethals	Per cent lethals	Number of lethals associated with rearrangements / Number of lethals tested	Correction factor for population	Per cent. association in correction population
Control	390	1	$.26 \pm .26$	0/1	0	0
1,000r	746	20*	$2.68 \pm .59$	2/18	18/20	$.30 \pm .21$
5,000r	319	41†	12.85 ± 1.86	10/36	36/41	3.57 ± 1.30

* One (1) not checked.
† Five (5) not checked.

multiply the total population tested for lethals by 18/20 at 1,000r and by 36/41 at 5,000r. The correction factors account for the lethal cases that were lost before they could be tested for association with rearrangements. The per cent. of the corrected population showing association was $.30 \pm .21$ at 1,000r and 3.57 ± 1.30 at 5,000r. Since these numbers differ from one another by at least twice their standard errors their difference is a real one.

Discussion

The breakage hypothesis is commonly accepted as an explanation for the mechanism of x-ray action in the production of chromosomal rearrangements. What is the mechanism whereby recessive lethals are produced by x-rays? Lethals have been thought to originate in several different ways due to x-ray action.

According to one view lethals arise as intragenic changes expressed as point or gene mutations. These may be of two types:

unassociated with the breakage phenomenon or associated with breakage and occurring at the breakage point of rearrangements. Another view of lethal origin is that they are due to intergenic changes and are position effects. How well do experimental data support the concept of a multiple origin for recessive lethals?

If a considerable fraction of lethals are unassociated with breakage then there should be a random distribution between points of breakage in rearrangements and lethals. This is unsupported, since Oliver (1932) and Demerec (1937) show that in the great majority of cases where lethals and rearrangements occur simultaneously on the X chromosome the lethal is located at a point of breakage.

If a considerable fraction of lethals are due to position effects then since rearrangements increase exponentially so should lethals. But as Oliver (1932) and Timoféeff-Ressovsky (1939) have shown, lethals increase linearly with dosage.

Assuming the great majority of lethals arise only at breaks the association between lethals and rearrangements is easy to visualize. At low doses there will be a certain per cent. of breaks at which lethals will occur, but in only few nuclei will there be several breaks. Since the opportunities for rearrangements will be limited few lethals will become associated with rearrangements. At higher doses the number of nuclei which will have more than one break increases exponentially and, accordingly, the number of rearrangements will also increase exponentially. Since proportionately more breaks will go into rearrangements at higher doses than lower doses more lethals will become associated with rearrangements at high doses. The data obtained agree with that from other workers in demonstrating an increase in association with dosage.

It is possible to show the relationship between breaks, recessive lethals and rearrangements quantitatively if the assumption is made that broken chromosome ends recombine at random.

According to the laws of probability if two events occur independently of one another, the frequency that they will occur at the same time will be the product of their separate frequencies. Accordingly, if the frequency with which breaks (B) will be recessive lethals (L) is multiplied by the frequency with which breaks will enter an observed rearrangement (R) then the product will be the frequency that the same number of breaks will have a recessive lethal and also have accomplished an observed

rearrangement (LR). Thus: $\frac{L}{B} \cdot \frac{R}{B} = \frac{LR}{B}$. Employing available data on L, R and LR it is possible to calculate the number of breaks involved. The relationship between them for the X chromosome is summarized in Table 2. The values for L came from the plot of data from Oliver (1932), Timoféeff-Ressovsky (1939) and the author corrected for cases where two lethals are produced on the same X chromosome. The figures for LR came from data of Oliver (1932) and the author corrected for cases where two lethals are present on the same X chromosome with both of them associated with rearrangements. The data from Bauer, Demerec and Kaufmann (1938) and Bauer (1939) were multiplied by .37, the fraction of detectable rearrangements involving the X chromosome in a genetic study as determined from the work of Patterson *et al.* (1934), and when extrapolated to 6,000r gave the values for R.

TABLE 2

SUMMARY OF RELATIONSHIP FOUND TO EXIST BETWEEN BREAKS, OBSERVED RECESSIVE LETHALS, OBSERVED REARRANGEMENTS AND ASSOCIATED LETHALS AND REARRANGEMENTS ON THE BASIS OF THE BREAKAGE HYPOTHESIS

	Per 100 X Chromosomes			
Dose × 1,000r	Number of breaks postulated (B)	Number of lethals observed (L)	Number of observed rearrangements (R)	Total observed associated lethals and rearrangements (LR)
1	10	3.1	0.9	0.30
2	20	6.2	3.7	1.11
3	30	9.3	6.9	2.14
4	40	12.4	11.9	3.73
5	50	15.5	16.5	5.14
6	60	18.6	18.0	5.50

The linear increase of breaks with dosage, expected in the usual concept of the breakage hypothesis, is confirmed in Table 2. The table shows the chance of breaks having a recessive lethal to be about 31 per cent. and that about 31 per cent. of rearrangements have lethals associated with them even though the dosage changes. Catcheside and Lea (1945) report similar values.

These data indicate that the great majority of x-ray-induced recessive lethals are probably produced at points of breakage and relegate to position effect and non-breakage mutation a relatively small part in the production of recessive lethals.

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LITERATURE CITED

Bauer, H.
1939. *Chromosoma,* 1: 343–390.
Bauer, H., M. Demerec and B. P. Kaufmann.
1938. *Genetics,* 23: 610–630.
Catcheside, D. G. and D. E. Lea.
1945. *Jour. Genet.,* 47: 10–24.
Demerec, M.
1937. *Cytologia,* Fujii Jubilee Volume: 1125–1132.
Oliver, C. P.
1932. *Zeit. f. ind. Abst. u. Vererb.,* 61: 447–488.
Patterson, J. T., W. Stone, S. Bedichek and M. Suche.
1934. Am. Nat., 68: 359–369.
Timoféeff-Ressovsky, N. W.
1939. *Chromosoma,* 1: 310–316.

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